INTERNATIONAL CONFERENCE ON
CHEMICAL AND BIOLOGICAL SCIENCES

SEPTEMBER 20-21, 2022

UMARU MUSA YAR'ADUA UNIVERSITY,
NIGERIA

PROCEEDINGS BOOK

Edited by
Dr. Yusuf Hassan
Dr. Kader DAĞCI KIRANŞAN

ISBN: 978-625-8246-08-7

INTERNATIONAL CONFERENCE ON CHEMICAL AND BIOLOGICAL SCIENCES

SEPTEMBER 20-21, 2022

UMARU MUSA YAR'ADUA UNIVERSITY, NIGERIA

PROCEEDINGS BOOK

Edited by
Dr. Yusuf Hassan
Dr. Kader DAĞCI KIRANŞAN

All rights of this book belongs to İKSAD Publishing House
Without permission can't be duplicated or copied.
Authors of chapters are responsible both ethically and juridically.
www.iensci.org

Issued: 15.10.2022

ISBN: 978-625-8246-08-7

CONFERENCE ID
INTERNATIONAL CONFERENCE ON CHEMICAL AND BIOLOGICAL SCIENCES

DATE AND PLACE
SEPTEMBER 20-21, 2022
UMARU MUSA YAR'ADUA UNIVERSITY, NIGERIA
Virtual and in person participation

ORGANIZED BY
İnstitute of Economic Development & Social Research, Turkey
Umaru Musa Yar'adua University, Nigeria

HEAD OF CONFERENCE
Dr. Yusuf Hassan

ORGANIZING BOARD
Dr. Yusuf Hassan - Umaru Musa Yar'adua University, Katsina, Nigeria
Dr. Aliyu D. Mohammed - Umaru Musa Yar'adua University, Katsina, Nigeria
Dr. Roumanatou S. Nassirou - Abdou Moumouni University, Niamey, Niger Republic
Dr. Lawaly M. Manzo - Abdou Moumouni University, Niamey, Niger Republic
Dr. Adama M. Sakho - Gamal Abdel Nasser University, Conakry, Guinea
Dr. Aboubacar Diallo - Gamal Abdel Nasser University, Conakry, Guinea
Dr. Osvaldo A.L. Ortet - University of Cape Verde, Praia, Cape Verde
Dr. Mara Y.S.D. Fernandes - University of Cape Verde, Praia, Cape Verde
Dr. Murat KIRANŞAN - Gumushane University, Turkey

COORDINATOR
Gulnaz GAFUROVA

NUMBER OF PAPERS FROM TURKEY
15

OTHER COUNTRIES
36

EVALUATION PROCESS
All submissions have undergone a double-blind peer review process

SCIENTIFIC BOARD

Professor Abdulhamid Ahmed - Umaru Musa Yar'adua University, Katsina, Nigeria
Prof. Dr. Arzu GÖRMEZ - Erzurum Technical University, Turkey
Assoc. Prof. Dr. hab. Elżbieta PATKOWSKA - University of Life Sciences in Lublin
Assoc. Prof. Dr. Betül APAYDIN YILDIRIM - Ataturk University, Turkey
Dr. Samaila M. Batagarawa - Umaru Musa Yar'adua University, Katsina, Nigeria
Dr. Bashir Abdulkadir - Umaru Musa Yar'adua University, Katsina, Nigeria
Dr. Aminu Usman - Umaru Musa Yar'adua University, Katsina, Nigeria
Professor Rabbani Adamou - Abdou Moumouni University, Niamey, Niger Republic
Dr. Alfa Keita - Abdou Moumouni University, Niamey, Niger Republic
Dr. Idrissa Moussa- Abdou Moumouni University, Niamey, Niger Republic
Dr. Abdoulaye Keita - Gamal Abdel Nasser University, Conakry, Guinea
Dr. Serwee Joe-Wia Faeflen - African Methodist Episcopal University, Monrovia, Liberia
Dr. Nare R.W. Alice - Institute of Research in Applied Science and Technology, Ouagadougou, Burkina Faso
Dr. Murat KIRANŞAN - Gumushane University, Turkey

PHOTO GALLERY

PHOTO GALLERY

PHOTO GALLERY

PHOTO GALLERY

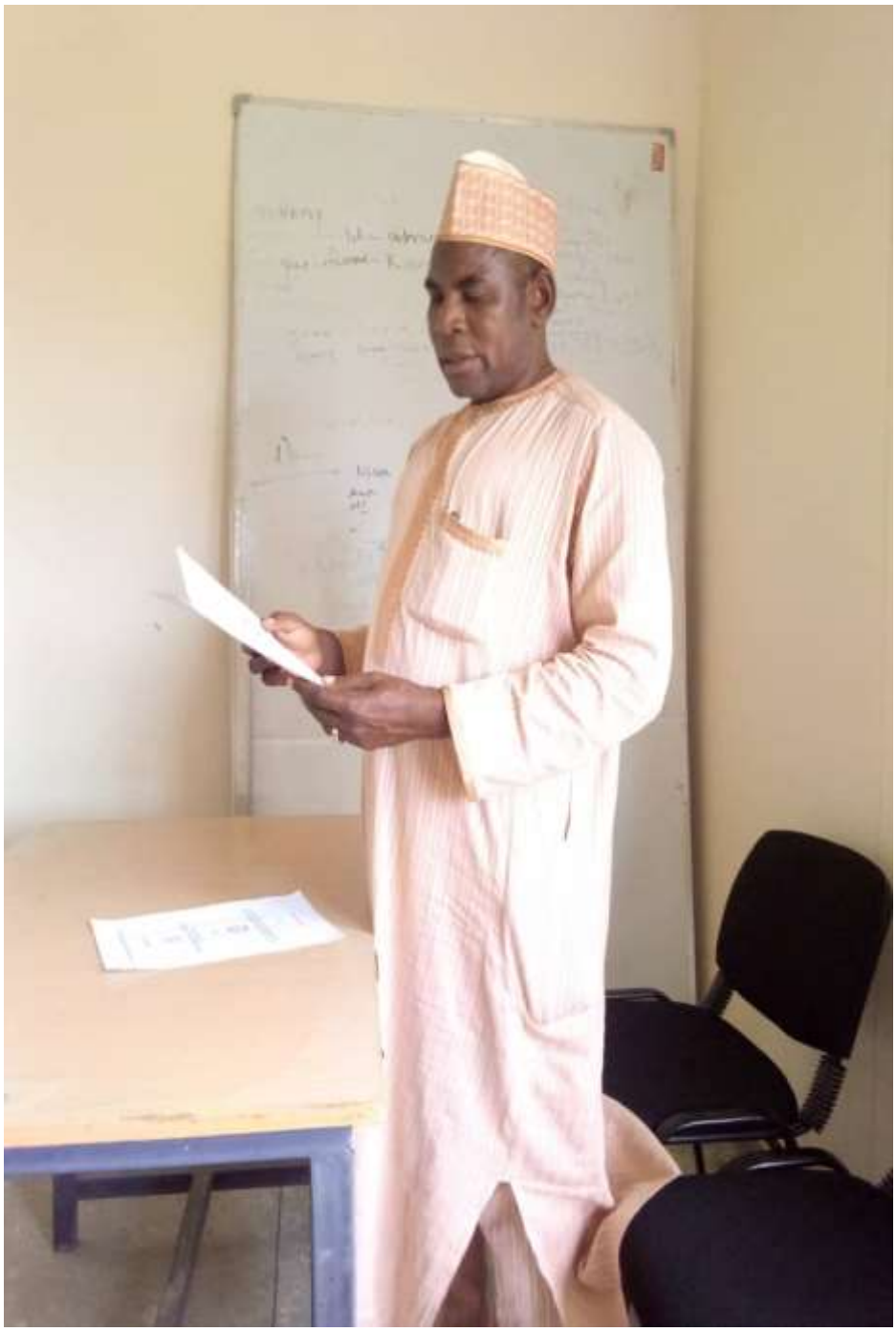


PHOTO GALLERY

PHOTO GALLERY

PHOTO GALLERY

PHOTO GALLERY

PHOTO GALLERY

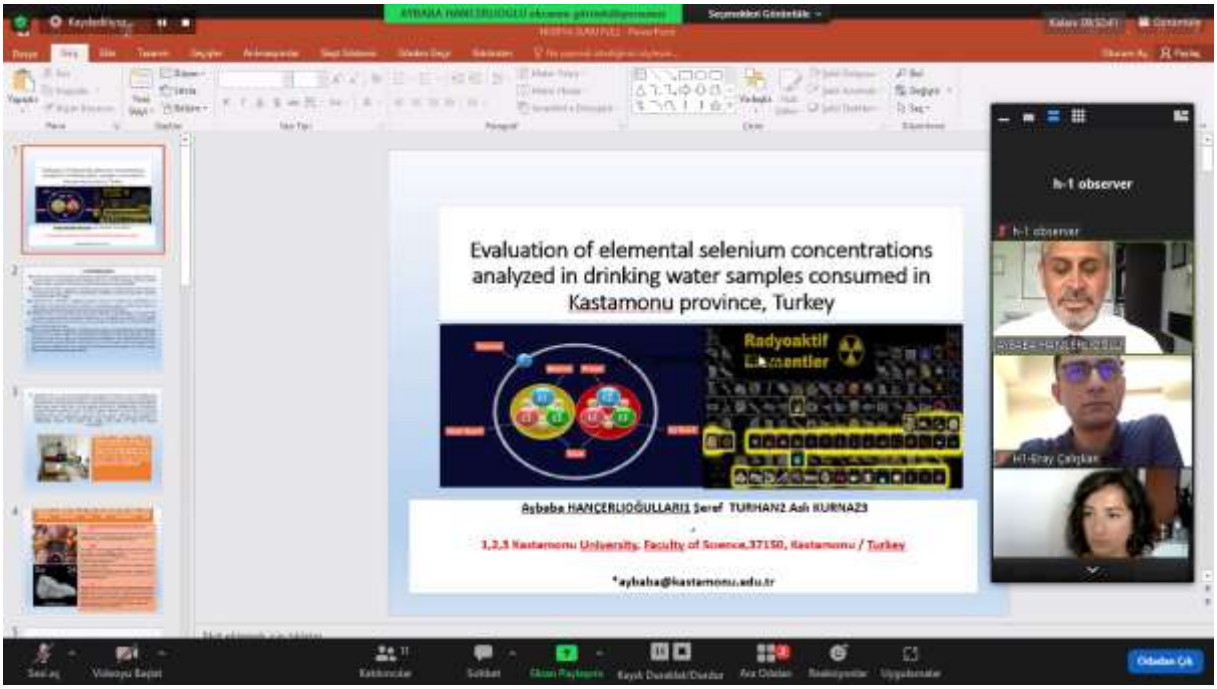
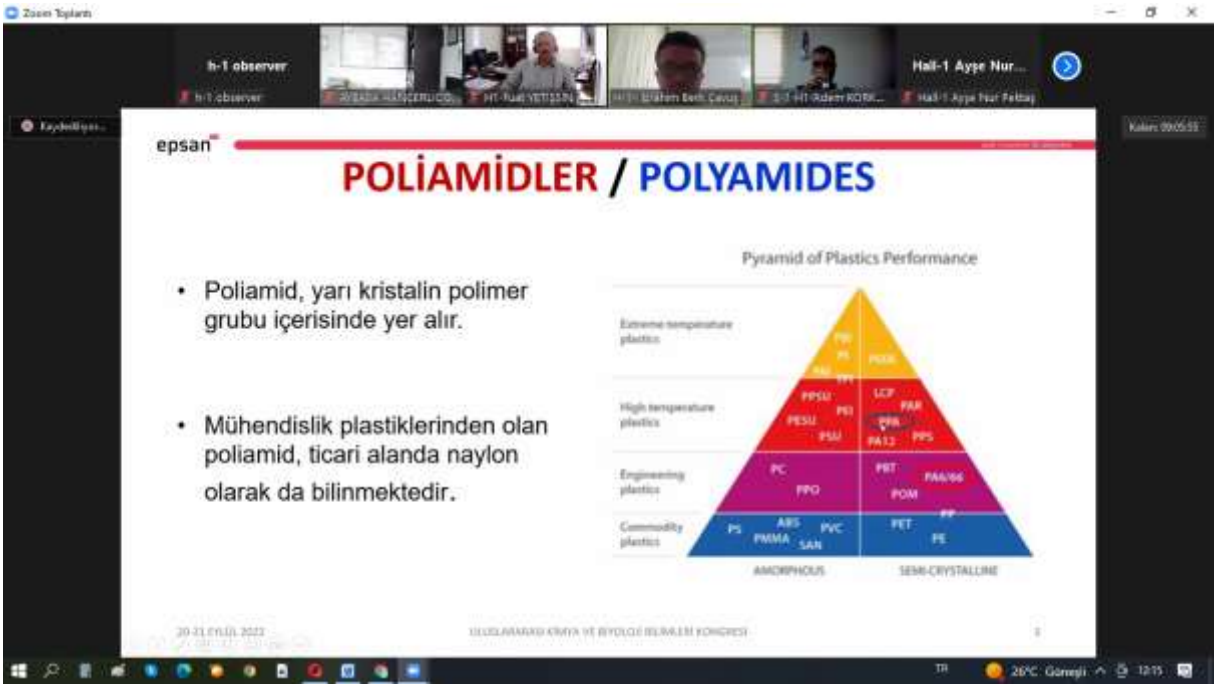


PHOTO GALLERY



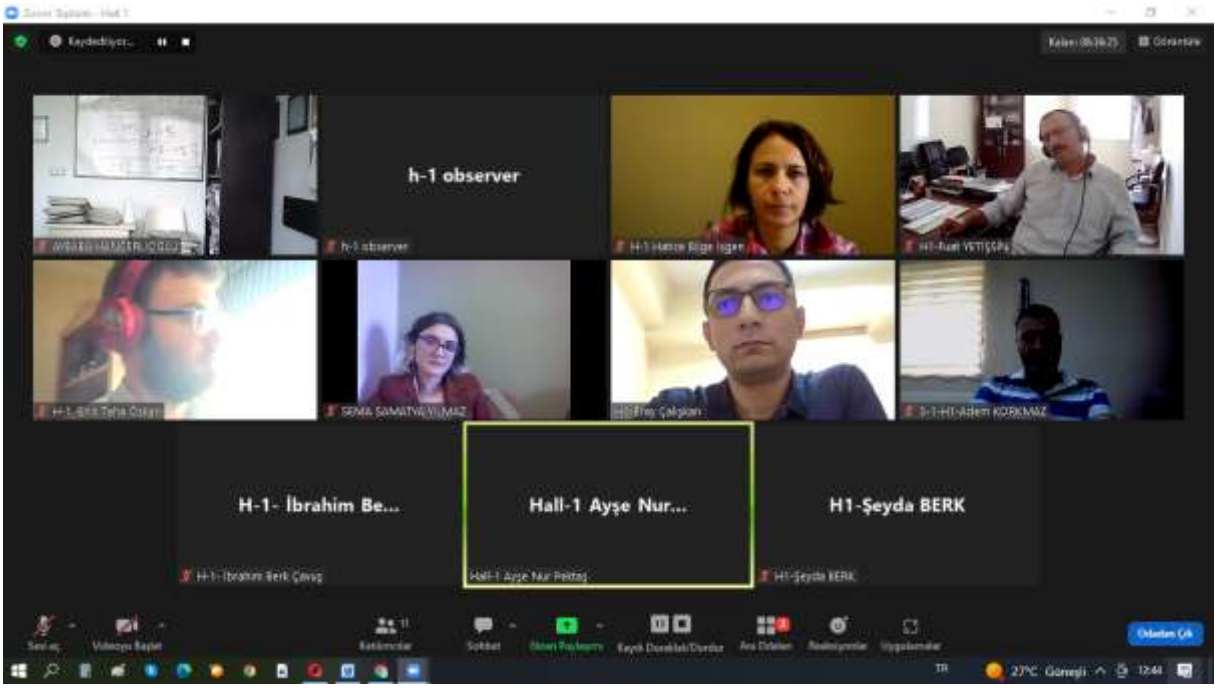


PHOTO GALLERY

PHOTO GALLERY

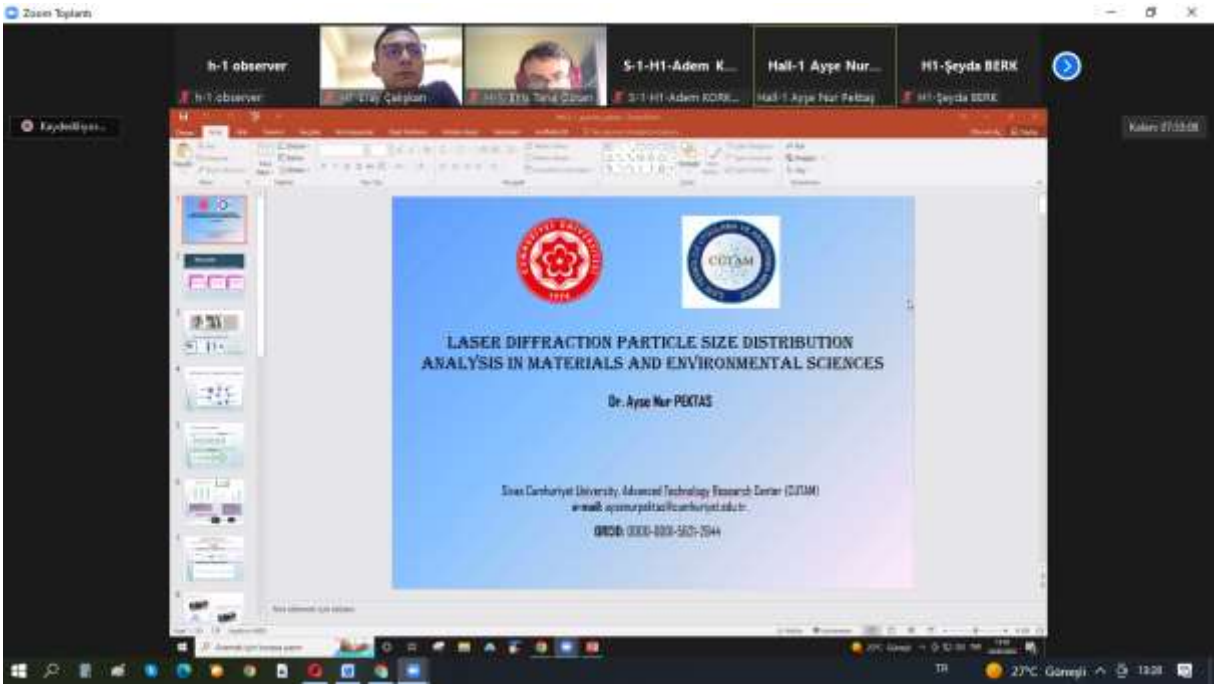
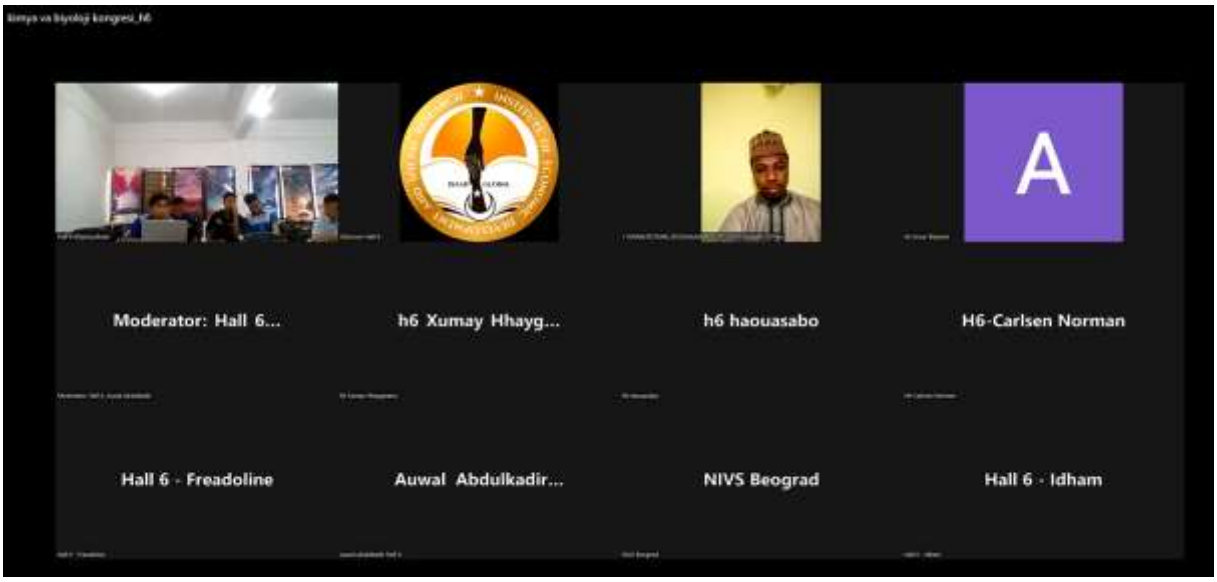


PHOTO GALLERY



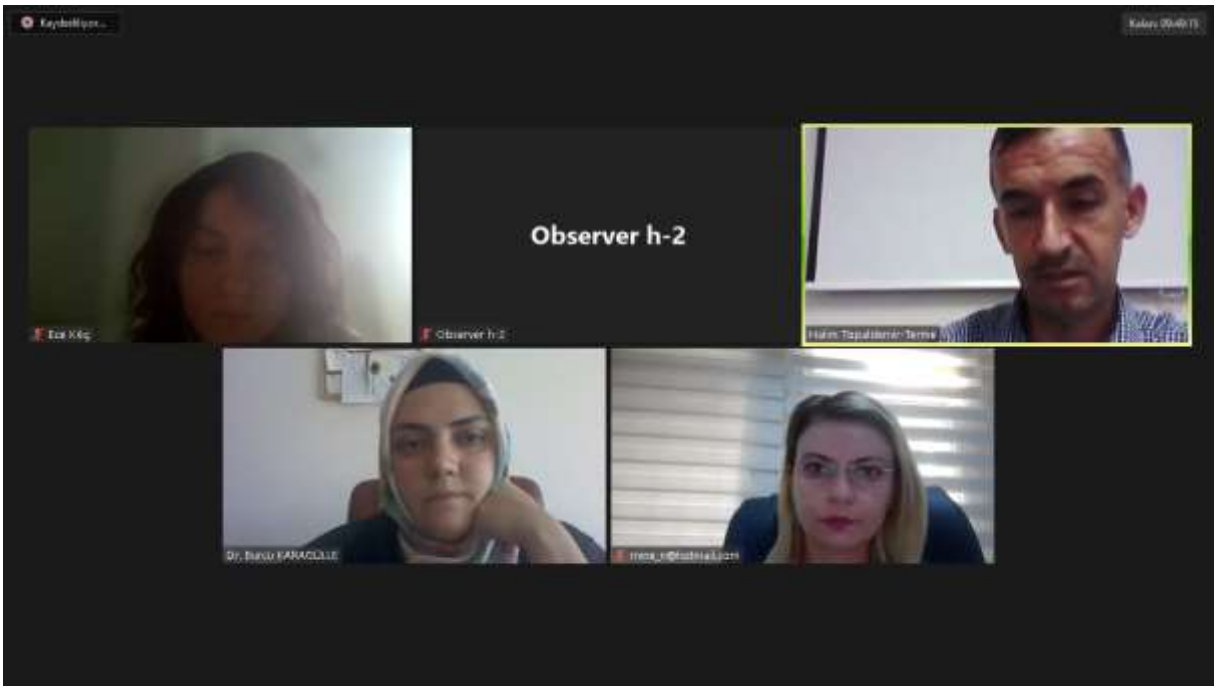


PHOTO GALLERY

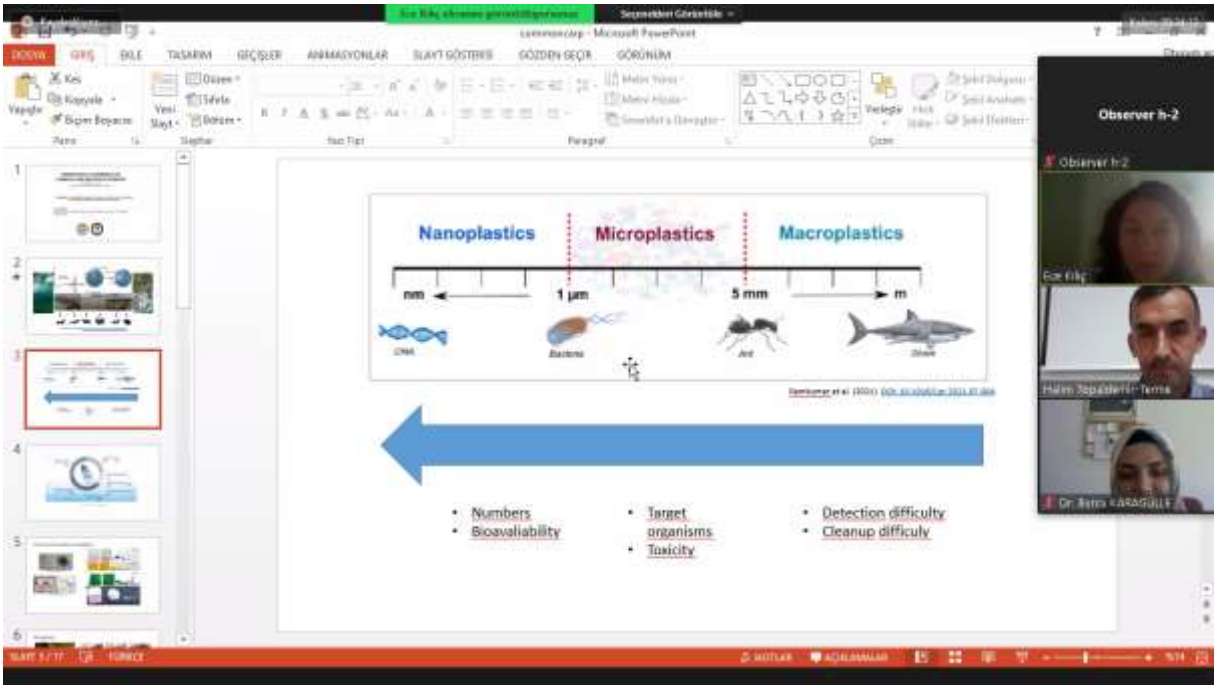


PHOTO GALLERY

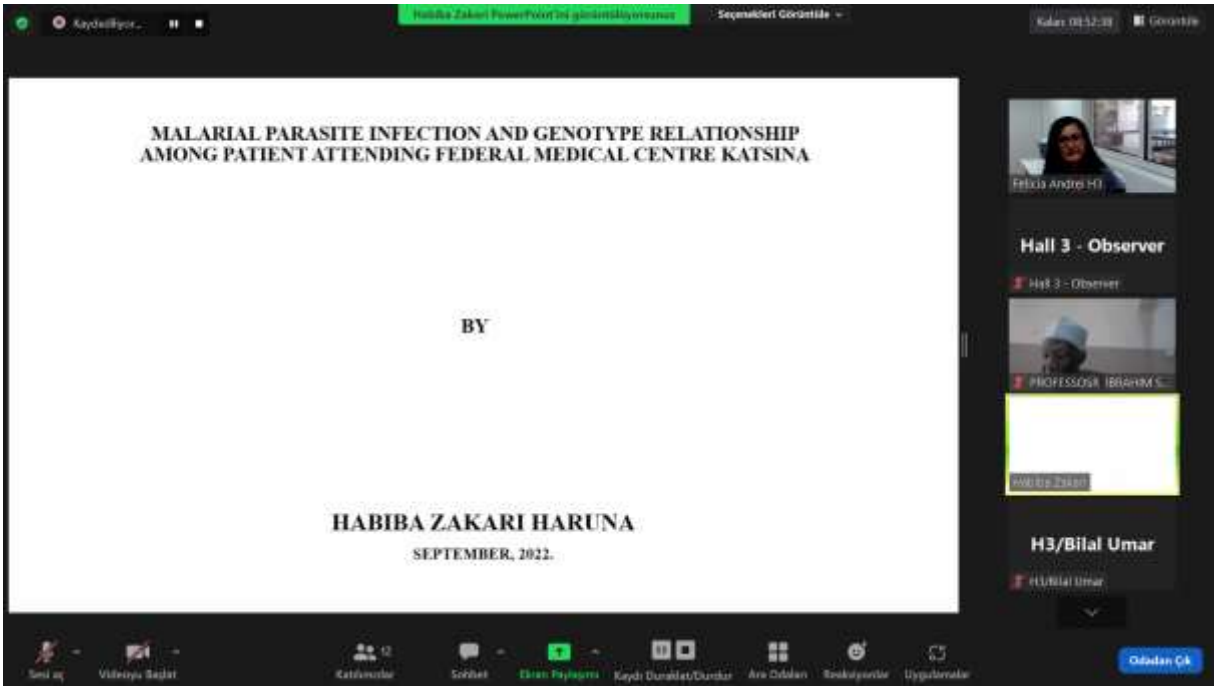


PHOTO GALLERY

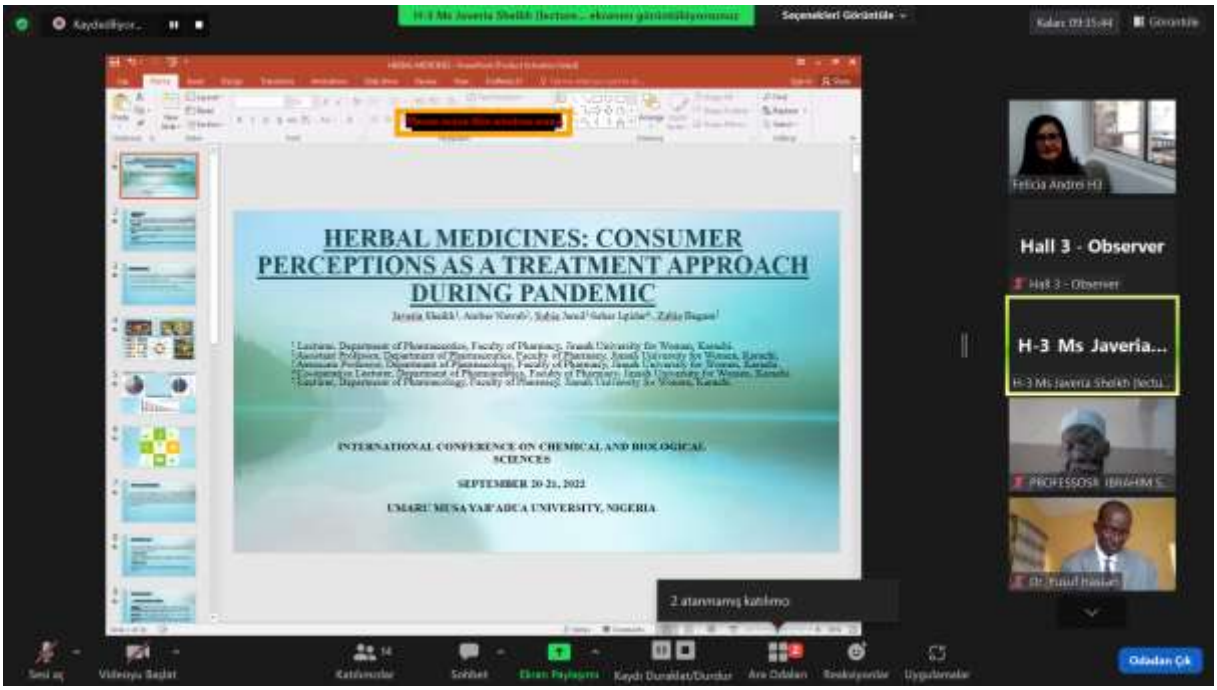


PHOTO GALLERY

INTERNATIONAL CONFERENCE ON CHEMICAL AND BIOLOGICAL SCIENCES

SEPTEMBER 20-21, 2022

UMARU MUSA YAR'ADUA UNIVERSITY, NIGERIA

CONFERENCE PROGRAM

Participant Countries: (11)

Nigeria, Türkiye, Malaysia, Kenya, Azerbaijan, Philippines, Serbia, Romania, Russia, United Kingdom, Pakistan

Önemli, Dikkatle Okuyunuz Lütfen

- Kongremizde Yazım Kurallarına uygun gönderilmiş ve bilim kurulundan geçen bildiriler için online (video konferans sistemi üzerinden) sunum imkanı sağlanmıştır.
- Online sunum yapabilmek için https://zoom.us/join sitesi üzerinden giriş yaparak "Meeting ID or Personal Link Name" yerine ID numarasını girerek oturuma katılabilirsiniz.
- Zoom uygulaması ücretsizdir ve hesap oluşturmaya gerek yoktur.
- Zoom uygulaması kaydolmadan kullanılabilir.
- Uygulama tablet, telefon ve PC'lerde çalışıyor.
- Her oturumdaki sunucular, sunum saatinden 5 dk öncesinde oturuma bağlanmış olmaları gerekmektedir.
- Tüm kongre katılımcıları canlı bağlanarak tüm oturumları dinleyebilir.
- Moderatör – oturumdaki sunum ve bilimsel tartışma (soru-cevap) kısmından sorumludur.

Dikkat Edilmesi Gerekenler - TEKNİK BİLGİLER

- Bilgisayarınızda mikrofon olduğuna ve çalıştığına emin olun.
- Zoom'da ekran paylaşma özelliğini kullanabilmelisiniz.
- Kabul edilen bildiri sahiplerinin mail adreslerine Zoom uygulamasında oluşturduğumuz oturuma ait ID numarası gönderilecektir.
- Katılım belgeleri kongre sonunda tarafınıza pdf olarak gönderilecektir.
- Kongre programında yer ve saat değişikliği gibi talepler dikkate alınmayacaktır.

Important, Please Read Carefully

- To be able to attend a meeting online, login via https://zoom.us/join site, enter ID "Meeting ID or Personal Link Name" and solidify the session.
- The Zoom application is free and no need to create an account.
- The Zoom application can be used without registration.
- The application works on tablets, phones and PCs.
- The participant must be connected to the session 5 minutes before the presentation time.
- All congress participants can connect live and listen to all sessions.
- Moderator is responsible for the presentation and scientific discussion (question-answer) section of the session.

Points to Take into Consideration - TECHNICAL INFORMATION

- Make sure your computer has a microphone and is working.
- You should be able to use screen sharing feature in Zoom.
- Attendance certificates will be sent to you as pdf at the end of the congress.
- Requests such as change of place and time will not be taken into consideration in the congress program.

***

Zoom'a giriş yapmadan önce lütfen örnekteki gibi salon numaranızı, adınızı ve soyadınızı belirtiniz

Before you login to Zoom please indicate your hall number, name and surname

exp. H-5, Enis Taha ÖZKAN

-Opening Ceremony-

Date: 20.09.2022
Nigeria Local Time: 09:00 - 09:30
Ankara Local Time: 11:00 - 11:30

***

Professor Haoua S. Sabo
Honorary Board Member

***

Professor Ibrahim Sada
Honorary Board Member

***

Dr. Yusuf Hassan
Head of Organizing Board

***

Dr. Mustafa Latif EMEK
President of IKSAD Institute

FACE TO FACE PRESENTATIONS

21.09.2022

Nigeria Local Time	10 00 : 12 30	UMARU MUSA YAR'ADUA UNIVERSITY, NIGERIA	

HEAD OF SESSION: Professor Abdulhamid Ahmed

AUTHORS	AFFILIATION	TOPIC TITLE
Abdul, N.A Ado, A. Abdullahi, S.A	*Umaru Musa 'Yar'adua University* *Federal University Dutsinma*	MOLECULAR CHARACTERISATION OF BACTERIAL CONTAMINANTS FROM DRINKING WATER SOURCES WITHIN KATSINA METROPOLIS
Abdullahi, N. S. Abdul, N.A Abubakar, M.G. Saidu, Y. Bala A.Y	*Usmanu Danfodiyo University Sokoto* *Umaru Musa 'Yar'adua University*	TOXICOLOGICAL EVALUATION OF AQUEOUS FRACTION OF SOLANUM INCANUM ROOT IN ALBINO RATS
Nura, H.B. Abdulkadir B. Abdullahi, S.	*Umaru Musa Yar'adua University*	MOLECULAR DETECTION AND QUANTIFICATION OF CYTOMEGALOVIRUS AMONG NEWBORN BABIES WITHIN KATSINA METROPOLIS
Nasiru, A. Suleiman, M.	*Umaru Musa Yar'adua University*	ENTOMOCIDAL EFFECT OF SOME ESSENTIAL OILS: AN ALTERNATIVE TO SYNTHETIC PESTICIDES IN THE CONTROL OF DERMESTES MACULATUS DEGEER 1774 (COLEOPTERA: DERMESTIDAE)
Raubilu, I. A. Usman A.D. Abdul N.A Aisha A.S.	*Hassan Usman Katsina Polytechnic* *Bayero University Kano* *Umaru Musa Yar'adua University*	PRESERVATIVE ACTIVITY AND ACUTE TOXICITY (LD50) OF MORINGA OLEIFERA SEED OIL ON BOILED WHITE RICE
Ahmad, Z.M. Saleh, M.S. Salisu, A. Ilu, M.B.	*Umaru Musa Yar'dua University*	SYNTHESIS OF METAKAOLIN GEOPOLYMERS BY REPLACEMENT OF SODIUM SILICATE ACTIVATOR WITH GUINEA CORN HUSK ASH

ONLINE PRESENTATIONS

20.09.2022 / Hall-1

Nigeria Local Time: 09:30 - 12:00

Ankara Local Time: 11:30 - 14:00

Meeting ID: 845 1779 2503

Passcode: 202122

HEAD OF SESSION: Dr. Ayşe Nur PEKTAŞ

AUTHORS	AFFILIATION	TOPIC TITLE
Aybaba HANÇERLIOĞULLARI Şeref TURHAN Aslı KURNAZ	*Kastamonu University*	EVALUATION OF ELEMENTAL SELENIUM CONCENTRATIONS ANALYSED IN DRINKING WATER SAMPLES CONSUMED IN KASTAMONU PROVINCE, TURKEY
Dr. Ayşe Nur PEKTAŞ	*Sivas Cumhuriyet University*	LASER DIFFRACTION PARTICLE SIZE DISTRIBUTION ANALYSIS IN MATERIALS AND ENVIRONMENTAL SCIENCES
Asst. Prof. Dr. Fuat YETİŞSİN	*Muş Alparslan University*	INVESTIGATION OF THE EFFECTS OF ACETONE O-(2-NAPHTHYLSULFONYL)OXIME PRETREATMENT ON BIOCHEMICAL PARAMETERS OF MAİZE SEEDLINGS UNDER DROUGHT STRESS
Dr. Adem KORKMAZ	*Muş Alparslan University*	SYNTHESIS, CHARACTERIZATION, AND MOLECULAR DOCKING STUDIES OF THE NOVEL ARYL NITRO-SULFONATE HYBRID COMPOUND BEARING AZOMETHINE
Research and Innovation engineer İbrahim Berk ÇAVUŞ	*Bursa Technical University*	OBTAINING IMPROVED SURFACE APPEARANCE OF SEMI AROMATIC POLYAMIDE 66 BLENDS
MSc., Hatice Bilge İŞGEN Dr., Sema SAMATYA YILMAZ Prof. Dr., Ayşe AYTAÇ	*Kocaeli University*	PRODUCTION AND CHARACTERIZATION OF HOLLOW POLYBUTYLENE SUCCINATE (PBS)/THERMOPLASTIC POLYURETHANE (TPU) NANOFIBERS
Dr. Eray ÇALIŞKAN	*Bingol University*	SYNTHESIS, CHARACTERIZATION AND DIELECTRIC PROPERTIES OF NOVEL METHACRYLATE HOMOPOLYMER WITH DIPEPTIDE SIDE CHAIN
Enis Taha ÖZKAN Prof. Dr. Yusuf KALENDER	*Gazi University*	PROTECTIVE ROLE OF GALLIC ACID ON FENITROTHION INDUCED TESTICULAR TOXICITY IN RATS
Asst. Prof. Dr. Şeyda BERK	*Sivas Cumhuriyet University*	mTOR SIGNALING PATHWAY AND ITS ROLE IN COLORECTAL CANCER TREATMENT

ONLINE PRESENTATIONS

20.09.2022 / Hall-2

Nigeria Local Time: 09:30 - 12:00

Ankara Local Time: 11:30 - 14:00

Meeting ID: 845 1779 2503

Passcode: 202122

HEAD OF SESSION: Dr. Halim TOPALDEMİR

AUTHORS	AFFILIATION	TOPIC TITLE
Dr. Halim TOPALDEMİR Prof. Dr. Beyhan TAŞ	*Ordu University*	IMPACT OF CLIMATE CHANGE ON WETLAND ECOSYSTEMS: AN INVESTIGATION IN SHALLOW WETLANDS IN YEŞILIRMAK DELTA
Dr. Ece KILIÇ	*Iskenderun Technical University*	INVESTIGATION OF BIOINDICATOR POTENTIAL OF COMMON CARP FOR THE MONITORING OF MICROPLASTIC POLLUTION IN FRESHWATER ENVIRONMENTS
S.B. BAGIROVA M.Y. HASANOVA N.F. SHUKUROVA L.A. ATAYEVA	*Institute of Dendrology, Azerbaijan*	INTRODUCTION AND PRODUCTIVITY OF STYPHNOLOBİUM JAPONİCUM (L.) SCHOTT STYPHNOLOBIUM JAPONICUM (L.) SCHOTT IN THE ABSHERON PENINSULA
Ph.D. Minare HASANOVA Aynur HUSEYNOVA Leyla ATAYEVA Saadat ALIYEVA Nigar BAFALZADE	*Institute of Dendrology of ANAS*	ECONOMIC EFFICIENCY OF THE LEUCAENA PULVERULENTA BENTH. TYPE IN AGRICULTURE
Assoc. Prof. Dr. Emine NAKİLCİOĞLU	*Ege University*	FLAVOR COMPOUNDS IN ONION AND GARLIC
Assoc. Prof. Dr. Emine NAKİLCİOĞLU	*Ege University*	POLYPHENOL OXIDASE (PPO) ENZYME AND INHIBITION METHODS IN FOODS
Assoc. Prof. MAMMADOVA K.A	*Azerbaijan State Pedagogical University*	EX SITU SEASONAL DEVELOPMENT OF SPECIES OF CARPINUS L. AND CORYLUS L. GENUS DISTRIBUTED IN THE NORTH-EASTERN PART OF THE GREATER CAUCASUS (AZERBAIJAN)
MAHMUDOV Yusif Magommed oglu	----	AZƏRBAYCANIN DAĞ EKOSİSTEMİ XÜSUSİ QORUNAN ƏRAZİLƏRİN NÜMUNƏSİNDƏ
Dr. Burcu KARAGULLE Dr. Tutku Can ACISU	*Firat University*	THE DETECTION AND MOLECULAR CHARACTERIZATION OF FUNGAL AND BACTERIAL AGENTS ISOLATED FROM PREPUTIUM INFECTION IN A DOG

ONLINE PRESENTATIONS

20.09.2022 / Hall-3

Nigeria Local Time: 09:30 - 12:00

Ankara Local Time: 11:30 - 14:00

Meeting ID: 845 1779 2503

Passcode: 202122

HEAD OF SESSION: Assist Prof. Dr. Felıcıa ANDREI

AUTHORS	AFFILIATION	TOPIC TITLE
Dr. Subia Jamil Amber Nawab Salma jabbar Simran Haresh kumar Wajeeha samar	*Jinnah University for women. Karachi Pakistan*	GLP AGONIST – AN INNOVATIVE APPROACH TO TREAT TYPE 2 DIABETES MELLITUS
Assistant Professor, Amber Nawab Lecturer, Javeria Sheikh Associate Professor, Subia Jamil Corporate Lecturer, Sehar Iqtidar Lecturer, Zubia Begum	*Jinnah University for women. Karachi Pakistan*	HERBAL MEDICINES: CONSUMER PERCEPTIONS AS A TREATMENT APPROACH DURING PANDEMIC
Sama SULTAN Harun ÖNLÜ Özlem OSMANAĞAOĞLU	*Ankara Universty* *Muş Alparslan University*	DETERMINATION OF RIBOFLAVIN PRODUCTION OF LACTOCOCCUS LACTIS MH3 AND PEDIOCOCCUS ACIDILACTICI MH10
Assist Prof. Dr. Felıcıa ANDREI Assist. Prof. Dr. Toma Ana Olivia Assist. Prof. Dr. Avram Ștefania Assist. Prof. Dr. Popa Valentin Tudor Lecturer Dr. Gencia Ioana Assoc. Prof. Dr. Cristodor Patricia Liana	*University of Medicine and Pharmacy "Victor Babes"*	UNIVERSITY INTERDISCIPLINARY COLLABORATIVE METHODS IN THE FIELD OF DERMATOLOGY
Habiba Zakari Haruna	*Umaru Musa Yar'adua University*	MALARIAL PARASITE INFECTION AND GENOTYPE RELATIONSHIP AMONG PATIENT ATTENDING FEDERAL MEDICAL CENTRE KATSINA
Syeda Kahkashan Kazmi Naheed Kauser Ayisha Aman Aqsa Idrees Rashida Rahmat Zohra Anoosha Sohail Saima Komal	*Jinnah University for women. Karachi Pakistan*	ISOLATION OF SKIN INFECTIOUS BACTERIA, ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS AGAINST Staphylococcus aureus, Staphylococcus epidermidis and E.coli STRAINS
Bilal Umar Yusuf Hassan Abdulhamid Ahmed	*Umaru Musa Yar'adua University*	BENZYL ACETATE DERIVATIVES: SYNTHESIS AND ANTIBACTERIAL ACTIVITY

ONLINE PRESENTATIONS

20.09.2022 / Hall-4

Nigeria Local Time: 09:30 - 12:00

Ankara Local Time: 11:30 - 14:00

Meeting ID: 845 1779 2503

Passcode: 202122

HEAD OF SESSION: Dr. Ahmed Salisu

AUTHORS	AFFILIATION	TOPIC TITLE
Dogun Ojochogu Emmamoge Orewere Ogenyi Ruth Ajibola Longchi Satkat Zacheaus	*Federal College of Forestry, Jos, Nigeria* *University of Jos, Nigeria*	ANTI-OXIDANT AND ANTI-BACTERIAL PROPERTIES OF Beta vulgaris L (BEET ROOT) ROOT EXTRACT
R. K Mustapha O. A. Adoum S. Y. Mudi M. Aliyu U.S. Abdussalam	*Yusuf Maitama Sule University* *Bayero University*	TOXICITY STUDIES AND IN VIVO ANTIPLASMODIAL ACTIVITIES OF LEAF AND STEM BARK EXTRACTS OF LANNEA MICROCARPA
Svjetlana Tesic Elena Kyrova Aleksandr Ignatov	*People's Friendship University of Russia* *Russian Institute of Plant Protection*	POSSIBLE SOURCES OF CLUSTERED REGULARLY INTERSPACED SHORT PALINDROMIC REPEATS IN Xanthomonas SPECIES
Maryam Saddiq Ahmed Salisu Kamaluddeen Kabir Yusuf Hassan	*Umaru Musa Yar'adua University*	PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL PROPERTIES OF NYMPHAEA LOTUS L. LEAVES EXTRACTS
Nafisatu A. Muhammad Abdullahi J. Muhammad Fatima Idris Sambo	*Yusuf Maitama Sule University* *Kano University of Science and Technology*	INSECTICIDAL ACTIVITY OF ENDOPHYTIC FUNGAL EXTRACTS FROM Lantana camara LEAVES AGAINST MALARIA VECTOR Anopheles gambiae
Dayo, L. Yusuf	*Umaru Musa Yar'adua University*	CHELATE ASSISTED PHYTOREMEDIATION OF LEAD AND COPPER FROM SOIL IRRIGATED WITH MUNICIPAL WASTE WATER USING JATROPHA CURCAS
Dayo, L. Yusuf Samaila, M. Batagarawa Ibrahim Sada	*Umaru Musa Yar'adua University*	PHYTOREMEDIATION OF LEAD AND CHROMIUM CONTAMINATED SOIL USING JATROPHA CURCAS

ONLINE PRESENTATIONS

20.09.2022 / Hall-5

Nigeria Local Time: 09:30 - 12:00

Ankara Local Time: 11:30 - 14:00

Meeting ID: 845 1779 2503

Passcode: 202122

HEAD OF SESSION: Dr. Aminu Usman

AUTHORS	AFFILIATION	TOPIC TITLE
Agada F.O Itodo A.U PhD Obinna O.O PhD Audu S.S PhD Shaibu B.	*J.S Tarka Uiversity Nigeria* *Nasarawa State University*	UV-VISIBLE SPECTROPHOTOMETRIC DETERMINATION OF DIETARY IRON IN BEANS, EGG, FISH AND PORK SOLD IN UNIVERSITY OF AGRICULTURE. MAKURDI NIGERIA
Ivan PAVLOVIC Milan STEVANOVIC Nemanja ZDRAVKOVIC Narcisa MEDERLE Aleksandra TASIC	*Scientific Institute of Veterinary Medicine of Serbia* *Academy of Beekeeping and Apitherapy of Serbia* *Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara*	SEPTICEMIA OF BEES
U.A. Sabiu G.O. Adejo A.S., Idoko N. Lawal Z. S. Abubakar	*Federal University Dutsin-Ma* *Umaru Musa Yaradua University*	AMELIORATIVE EFFECT OF ALLIUM CEPA AND CURCUMA LONGA POWDER ON LIVER AND KIDNEY FUNCTION INDICES OF MALE ALBINO RATS EXPOSED TO 2,3,7,8-TERACHOLORODIBENZO-P-DIOXIN
Usman, A. Abubakar, M.	*Umaru MusaYaradua University*	SERUM GLUCOSE LEVELS, LIVER AND KIDNEY FUNCTION INDICES IN ALLOXAN INDUCED DIABETIC RATS SUPPLEMENTED WITH AQUEOUS EXTRACT OF HELIXANTHERA PARASITICA LEAVES
Oyedum, Uche Mary Agbala, J.	*Federal University of Technology, Minna*	ISOLATION OF MULTIDRUG RESISTANT BACTERIA FROM TWO LOCALLY PRODUCED DRINKS SOLD IN NORTH CENTRAL, NIGERIA
Romar A. Alegado Ma. Shiela G. Mendoza Ronel S. De Guzman Myla G. Santiago	*President Ramon Magsaysay State University*	NITROUS OXIDE EMISSION ON N-FERTILIZATION IN RICE GROWN UNDER PRMSU-SM CONDITION

ONLINE PRESENTATIONS

20.09.2022 / Hall-6

Nigeria Local Time: 09:30 - 12:00

Ankara Local Time: 11:30 - 14:00

Meeting ID: 845 1779 2503

Passcode: 202122

HEAD OF SESSION: Dr. Auwal Abdulkadir

AUTHORS	AFFILIATION	TOPIC TITLE
Dr. Osman Y. Yansaneh	*University of Hull*	A TECHNICAL STUDY ON PLUG FLOW REACTOR DESIGN AND EXPERIMENTATION FOR WASTE PLASTIC PYROLYSIS TO VALUABLE MATERIALS
Xumay B. Hhaygwawu Achisa C. Mecha Jacquiline K. Makatiani	*Moi University*	OPTIMIZATION OF BIOGAS PRODUCTION THROUGH CO-DIGESTION OF ORGANIC WASTE FROM HUMAN BODY
Abdulkadir HALLIRU	*Umaru Musa Yaradua University*	PROLIFERATION OF SYNTHETIC AGROCHEMICALS IN NIGERIA: IMPLICATION ON THE GOAL 14 TH AND 15 TH OF SUSTAINABLE DEVELOPMENT
Auwal Abdulkadir Mohd Zamir Pakhuruddin	*Umaru Musa Yar'adua University* *Universiti Sains Malaysia*	EFFECT OF HYDROFLUORIC ACID CONCENTRATION ON MORPHOLOGICAL AND OPTICAL PROPERTIES OF BLACK SILICON FABRICATED BY ONE-STEP ELECTROLESS SILVER-ASSISTED WET CHEMICAL ETCHING FOR SOLAR CELLS
Carlsen Norman Idham Sahaidainain Khairisyafwan Waimin Muhammad Zulkarnain Muhammad Nazlie Ernard Losumin	*Keningau Vocational College*	UPGRADING SCISSOR CAR JACK WITH BATTERY TO REDUCE PHYSICAL LABOR
Ansar Bilyaminu Adam Aliyu Danmusa Mohammed	*Federal University Wukari,Taraba Nigeria* *Umaru Musa Yar'adua University*	EXTRACTION AND MODIFICATION OF CELLULOSE FROM CALOTROPIS PROCERA PLANT
Ansar Bilyaminu Adam Aliyu Danmusa Mohammed Aminu Ado Kaugama	*Federal University Wukari,Taraba Nigeria* *Umaru Musa Yar'adua University*	WASTE WATER TREATMENT USING CELLULOSE CONTAINING LABILE CATIONIC GROUP

CONTENTS

CONFERENCE ID	I
PHOTO GALLERY	II
PROGRAM	III
CONTENT	IV

AUTHOR	TITLE	No
Abdul, N.A Ado, A. Abdullahi, S.A	MOLECULAR CHARACTERISATION OF BACTERIAL CONTAMINANTS FROM DRINKING WATER SOURCES WITHIN KATSINA METROPOLIS	1
Abdullahi, N. S. Abdul, N.A Abubakar, M.G. Saidu, Y. Bala A.Y	TOXICOLOGICAL EVALUATION OF AQUEOUS FRACTION OF SOLANUM INCANUM ROOT IN ALBINO RATS	9
Nura, H.B. Abdulkadir B. Abdullahi, S.	MOLECULAR DETECTION AND QUANTIFICATION OF CYTOMEGALOVIRUS AMONG NEWBORN BABIES WITHIN KATSINA METROPOLIS	21
Nasiru, A. Suleiman, M.	ENTOMOCIDAL EFFECT OF SOME ESSENTIAL OILS: AN ALTERNATIVE TO SYNTHETIC PESTICIDES IN THE CONTROL OF DERMESTES MACULATUS DEGEER 1774 (COLEOPTERA: DERMESTIDAE)	30
Raubilu, I. A. Usman A.D. Abdul N.A Aisha A.S.	PRESERVATIVE ACTIVITY AND ACUTE TOXICITY (LD50) OF MORINGA OLEIFERA SEED OIL ON BOILED WHITE RICE	43
Ahmad, Z.M. Saleh, M.S. Salisu, A. Ilu, M.B.	SYNTHESIS OF METAKAOLIN GEOPOLYMERS BY REPLACEMENT OF SODIUM SILICATE ACTIVATOR WITH GUINEA CORN HUSK ASH	52
Aybaba HANÇERLIOĞULLARI Şeref TURHAN Aslı KURNAZ	EVALUATION OF ELEMENTAL SELENIUM CONCENTRATIONS ANALYSED IN DRINKING WATER SAMPLES CONSUMED IN KASTAMONU PROVINCE, TURKEY	53
Ayşe Nur PEKTAŞ	LASER DIFFRACTION PARTICLE SIZE DISTRIBUTION ANALYSIS IN MATERIALS AND ENVIRONMENTAL SCIENCES	60
Fuat YETİŞSİN	INVESTIGATION OF THE EFFECTS OF ACETONE O-(2-NAPHTHYLSULFONYL)OXIME PRETREATMENT ON BIOCHEMICAL PARAMETERS OF MAİZE SEEDLINGS UNDER DROUGHT STRESS	68
Adem KORKMAZ	SYNTHESIS, CHARACTERIZATION, AND MOLECULAR DOCKING STUDIES OF THE NOVEL ARYL NITRO-SULFONATE HYBRID COMPOUND BEARING AZOMETHINE	70
İbrahim Berk ÇAVUŞ	OBTAINING IMPROVED SURFACE APPEARANCE OF SEMI AROMATIC POLYAMIDE 66 BLENDS	73
Hatice Bilge İŞGEN Sema SAMATYA YILMAZ Ayşe AYTAÇ	PRODUCTION AND CHARACTERIZATION OF HOLLOW POLYBUTYLENE SUCCINATE (PBS)/THERMOPLASTIC POLYURETHANE (TPU) NANOFIBERS	75

Eray ÇALIŞKAN	SYNTHESIS, CHARACTERIZATION AND DIELECTRIC PROPERTIES OF NOVEL METHACRYLATE HOMOPOLYMER WITH DIPEPTIDE SIDE CHAIN	77
Enis Taha ÖZKAN Yusuf KALENDER	PROTECTIVE ROLE OF GALLIC ACID ON FENITROTHION INDUCED TESTICULAR TOXICITY IN RATS	78
Şeyda BERK	mTOR SIGNALING PATHWAY AND ITS ROLE IN COLORECTAL CANCER TREATMENT	80
Halim TOPALDEMİR Beyhan TAŞ	IMPACT OF CLIMATE CHANGE ON WETLAND ECOSYSTEMS: AN INVESTIGATION IN SHALLOW WETLANDS IN YEŞILIRMAK DELTA	89
Ece KILIÇ	INVESTIGATION OF BIOINDICATOR POTENTIAL OF COMMON CARP FOR THE MONITORING OF MICROPLASTIC POLLUTION IN FRESHWATER ENVIRONMENTS	99
S.B. BAGIROVA M.Y. HASANOVA N.F. SHUKUROVA L.A. ATAYEVA	INTRODUCTION AND PRODUCTIVITY OF STYPHNOLOBİUM JAPONİCUM (L.) SCHOTT STYPHNOLOBIUM JAPONICUM (L.) SCHOTT IN THE ABSHERON PENINSULA	105
Minare HASANOVA Aynur HUSEYNOVA Leyla ATAYEVA Saadat ALIYEVA Nigar BAFALZADE	ECONOMIC EFFICIENCY OF THE LEUCAENA PULVERULENTA BENTH. TYPE IN AGRICULTURE	106
Emine NAKİLCİOĞLU	FLAVOR COMPOUNDS IN ONION AND GARLIC	113
Emine NAKİLCİOĞLU	POLYPHENOL OXIDASE (PPO) ENZYME AND INHIBITION METHODS IN FOODS	117
MAMMADOVA K.A	EX SITU SEASONAL DEVELOPMENT OF SPECIES OF CARPINUS L. AND CORYLUS L. GENUS DISTRIBUTED IN THE NORTH-EASTERN PART OF THE GREATER CAUCASUS (AZERBAIJAN)	121
MAHMUDOV Yusif Magommed oglu	AZƏRBAYCANIN DAĞ EKOSİSTEMİ XÜSUSİ QORUNAN ƏRAZİLƏRİN NÜMUNƏSİNDƏ	125
Burcu KARAGULLE Tutku Can ACISU	THE DETECTION AND MOLECULAR CHARACTERIZATION OF FUNGAL AND BACTERIAL AGENTS ISOLATED FROM PREPUTIUM INFECTION IN A DOG	138
Subia Jamil Amber Nawab Salma jabbar Simran Haresh kumar Wajeeha samar	GLP AGONIST – AN INNOVATIVE APPROACH TO TREAT TYPE 2 DIABETES MELLITUS	140
Amber Nawab Javeria Sheikh Subia Jamil Sehar Iqtidar Zubia Begum	HERBAL MEDICINES: CONSUMER PERCEPTIONS AS A TREATMENT APPROACH DURING PANDEMIC	141
Sama SULTAN Harun ÖNLÜ Özlem OSMANAĞAOĞLU	DETERMINATION OF RIBOFLAVIN PRODUCTION OF LACTOCOCCUS LACTIS MH3 AND PEDIOCOCCUS ACIDILACTICI MH10	142
Felıcıa ANDREI Toma Ana Olivia Avram Ştefania Popa Valentin Tudor	UNIVERSITY INTERDISCIPLINARY COLLABORATIVE METHODS IN THE FIELD OF DERMATOLOGY	143

Gencia Ioana Cristodor Patricia Liana		
Habiba Zakari Haruna	MALARIAL PARASITE INFECTION AND GENOTYPE RELATIONSHIP AMONG PATIENT ATTENDING FEDERAL MEDICAL CENTRE KATSINA	144
Syeda Kahkashan Kazmi Naheed Kauser Ayisha Aman Aqsa Idrees Rashida Rahmat Zohra Anoosha Sohail Saima Komal	ISOLATION OF SKIN INFECTIOUS BACTERIA, ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS AGAINST Staphylococcus aureus, Staphylococcus epidermidis and E.coli STRAINS	145
Bilal Umar Yusuf Hassan Abdulhamid Ahmed	BENZYL ACETATE DERIVATIVES: SYNTHESIS AND ANTIBACTERIAL ACTIVITY	146
Dogun Ojochogu Emmamoge Orewere Ogenyi Ruth Ajibola Longchi Satkat Zacheaus	ANTI-OXIDANT AND ANTI-BACTERIAL PROPERTIES OF Beta vulgaris L (BEET ROOT) ROOT EXTRACT	147
R. K Mustapha O. A. Adoum S. Y. Mudi M. Aliyu U.S. Abdussalam	TOXICITY STUDIES AND IN VIVO ANTIPLASMODIAL ACTIVITIES OF LEAF AND STEM BARK EXTRACTS OF LANNEA MICROCARPA	153
Svjetlana Tesic Elena Kyrova Aleksandr Ignatov	POSSIBLE SOURCES OF CLUSTERED REGULARLY INTERSPACED SHORT PALINDROMIC REPEATS IN Xanthomonas SPECIES	154
Maryam Saddiq Ahmed Salisu Kamaluddeen Kabir Yusuf Hassan	PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL PROPERTIES OF NYMPHAEA LOTUS L. LEAVES EXTRACTS	155
Nafisatu A. Muhammad Abdullahi J. Muhammad Fatima Idris Sambo	INSECTICIDAL ACTIVITY OF ENDOPHYTIC FUNGAL EXTRACTS FROM Lantana camara LEAVES AGAINST MALARIA VECTOR Anopheles gambiae	156
Dayo, L. Yusuf	CHELATE ASSISTED PHYTOREMEDIATION OF LEAD AND COPPER FROM SOIL IRRIGATED WITH MUNICIPAL WASTE WATER USING JATROPHA CURCAS	157
Dayo, L. Yusuf Samaila, M. Batagarawa Ibrahim Sada	PHYTOREMEDIATION OF LEAD AND CHROMIUM CONTAMINATED SOIL USING JATROPHA CURCAS	158
Agada F.O Itodo A.U PhD Obinna O.O PhD Audu S.S PhD Shaibu B.	UV-VISIBLE SPECTROPHOTOMETRIC DETERMINATION OF DIETARY IRON IN BEANS, EGG, FISH AND PORK SOLD IN UNIVERSITY OF AGRICULTURE. MAKURDI NIGERIA	170
Ivan PAVLOVIC Milan STEVANOVIC Nemanja ZDRAVKOVIC Narcisa MEDERLE Aleksandra TASIC	SEPTICEMIA OF BEES	171
U.A. Sabiu G.O. Adejo A.S., Idoko N. Lawal Z. S. Abubakar	AMELIORATIVE EFFECT OF ALLIUM CEPA AND CURCUMA LONGA POWDER ON LIVER AND KIDNEY FUNCTION INDICES OF MALE ALBINO RATS EXPOSED TO 2,3,7,8-TERACHOLORODIBENZO-P-DIOXIN	172
Usman, A. Abubakar, M.	SERUM GLUCOSE LEVELS, LIVER AND KIDNEY FUNCTION INDICES IN ALLOXAN INDUCED DIABETIC RATS SUPPLEMENTED WITH AQUEOUS EXTRACT OF HELIXANTHERA PARASITICA LEAVES	182

Oyedum, Uche Mary Agbala, J.	ISOLATION OF MULTIDRUG RESISTANT BACTERIA FROM TWO LOCALLY PRODUCED DRINKS SOLD IN NORTH CENTRAL, NIGERIA	183
Romar A. Alegado Ma. Shiela G. Mendoza Ronel S. De Guzman Myla G. Santiago	NITROUS OXIDE EMISSION ON N-FERTILIZATION IN RICE GROWN UNDER PRMSU-SM CONDITION	191
Osman Y. Yansaneh	A TECHNICAL STUDY ON PLUG FLOW REACTOR DESIGN AND EXPERIMENTATION FOR WASTE PLASTIC PYROLYSIS TO VALUABLE MATERIALS	192
Xumay B. Hhaygwawu Achisa C. Mecha Jacquiline K. Makatiani	OPTIMIZATION OF BIOGAS PRODUCTION THROUGH CO-DIGESTION OF ORGANIC WASTE FROM HUMAN BODY	193
Abdulkadir HALLIRU	PROLIFERATION OF SYNTHETIC AGROCHEMICALS IN NIGERIA: IMPLICATION ON THE GOAL 14 TH AND 15 TH OF SUSTAINABLE DEVELOPMENT	208
Auwal Abdulkadir Mohd Zamir Pakhuruddin	EFFECT OF HYDROFLUORIC ACID CONCENTRATION ON MORPHOLOGICAL AND OPTICAL PROPERTIES OF BLACK SILICON FABRICATED BY ONE-STEP ELECTROLESS SILVER-ASSISTED WET CHEMICAL ETCHING FOR SOLAR CELLS	215
Carlsen Norman Idham Sahaidainain Khairisyafwan Waimin Muhammad Zulkarnain Muhammad Nazlie Ernard Losumin	UPGRADING SCISSOR CAR JACK WITH BATTERY TO REDUCE PHYSICAL LABOR	224
Ansar Bilyaminu Adam Aliyu Danmusa Mohammed	EXTRACTION AND MODIFICATION OF CELLULOSE FROM CALOTROPIS PROCERA PLANT	225
Ansar Bilyaminu Adam Aliyu Danmusa Mohammed Aminu Ado Kaugama	WASTE WATER TREATMENT USING CELLULOSE CONTAINING LABILE CATIONIC GROUP	231

MOLECULAR CHARACTERISATION OF BACTERIAL CONTAMINANTS FROM DRINKING WATER SOURCES WITHIN KATSINA METROPOLIS

Abdul, N.A, *
Ado, A.
Abdullahi, S.A
[1]Department of Microbiology, Umaru Musa 'Yar'adua University, PMB 2218, Katsina, Nigeria
[2]Department of Microbiology, Federal University Dutsinma, PMB 5001, Dutsin-Ma, Katsina State
[3]Department of Biochemistry, Umaru Musa 'Yar'adua University, PMB 2218, Katsina, Nigeria

ABSTRACT
Katsina Metropolis is witnessing poor quality of drinking water (sachet and tap water), due to inadequate/inefficient treatment plants, and failure to adhere to standards. The bacterial contaminants in poor drinking water can potentially cause waterborne diseases. Conventional methods of identifying bacteria can lead to identification failure, hence the need for molecular techniques. This study was carried to molecularly identify some common bacteria associated with sachet and tap water consumed within Katsina metropolis. Bacteriological examinations were carried out on 100 water samples (50 each for sachet and tap water) collected from five locations within Katsina metropolis, according to American Public Health Association protocols. Pure cultures of bacterial isolates were identified by amplifying the 16S rRNA gene using specific primers, after DNA extraction using ZymoresearchTM kit; thereafter, extracted DNA was amplified through PCR. The products were visualized on agarose gel electrophoresis, and the sequence was compared to NCBI BLAST to identify the bacteria. Phylogenetic tree was constructed using multiple sequence alignment as guided by EMBL database. Based on the BLAST results, the identified bacteria were *Enterobacter kobei* AA01 (97.86% similarity with *Enterobacter kobei* CIP 105566), and *Escherichia marmotae* AN01 (79.53% similarity with *Escherichia marmotae* HT073016). However, the second isolate needs further studies, e.g. DNA-DNA hybridization to ascertain its real identity. These bacteria are novel water contaminants, and this study is amongst the first to report their presence in drinking water, to the best of our knowledge. This study reported the presence of *E. kobei* and *E. marmotae* which are uncommon bacterial contaminants that contaminate sachet and tap water in the study area.
Keywords: Molecular identification, bacteria, drinking water, 16S rRNA gene

INTRODUCTION
Katsina Metropolis in particular, and other cities in Nigeria, and by extension, Africa; witness poor quality of drinking water (sachet and tap water), and this can be attributed to both inadequate treatment plants, inefficiency in the management of the pipeline systems for water distribution and ultimately failure to adhere to standards (Ayotunde *et al*., 2011; Aderibigbe et al., 2008).
On a global scale, up to 2 billion people are estimated to be lacking safe drinking water (WHO, 2014). Global statistics indicate that many people die from unsafe water than from war, with about 30,000 global weekly deaths attributed to unsuitable drinking water (Albertiri, 2018). Such unwholesome water is usually contaminated with bacteria, making it unsuitable for drinking and preparation of food. Pathogenic bacterial contaminants of water often

originate from fecal sources (Kumar and Xagoraraki, 2010), which may potentially cause waterborne diseases, morbidity and mortality.

Therefore, estimating the numbers and types of bacteria present in water is a key aspect of water quality known as bacteriological analysis of water, and by determining the bacterial contaminants of water, inferences can be drawn about the suitability of the water for use (Bernette *et al*., 2018). The preferable method of analysis is to look for indicator organisms which indicate water pollution (Ibemesim, 2014). These indicator organisms include non-specific coliforms such as *Escherichia coli* and faecal Streptococci such as *Enterococcus faecalis* that are very commonly found in the human or animal gut and which, if detected, may suggest the presence of sewage (Jay *et al*., 2015). Detecting these bacteria in water therefore serves as a direct indicator of the approximate level of pathogens in that water source.

From the foregoing, assessment of drinking-water is principally a health-based activity which is necessary for the protection of public health through ensuring that the water supplied is of a good quality, and this provides baseline data for the surveillance and strategic planning in the control and prevention of spread waterborne diseases (APHA, 2017; WHO, 2014).

Conventional methods employed in identifying the bacterial contaminants from water can lead to identification failure, hence the need for advanced techniques to accurately identify these bacteria, such as molecular techniques, in particular, 16S rRNA analysis, which is regarded as a gold standard in the identification of bacteria from water sources (Enaigbe *et al*., 2019; Olowe *et al*., 2017; Ghatak *et al*., 2013).

Therefore, this study was carried out to molecularly identify some common bacteria associated with sachet and tap water consumed within Katsina metropolis.

MATERIALS AND METHODS

Sample Collection

A total of 100 water samples, 10 each from sachet and tap water were collected using simple random sampling from five (5) different areas within Katsina Metropolis, namely: Kofar Kaura, Kofar Kwaya, Kofar Marusa, Kofar Durbi and Kofar Keke, according to the protocols of the American Public Health Association (APHA, 2017).

The tap water samples were collected in sterile screw capped 100ml capacity bottles; packaged in an ice-packed cooled box and then transported to Microbiology Laboratory, Department of Microbiology, Umaru Musa Yaradua University Katsina for analyses.

Conventional Isolation, Identification and Characterization of the Bacteria Present in the Water Samples

The bacteria present in the water samples were first characterized using the conventional methods of: (i). serial dilution, (ii). inoculation on suitable media, (iii). culture and incubation, (iv). Gram's staining and (v). biochemical tests.

Serially dilution was carried out up to the 10^{-6} dilution according to the procedure adopted from Willey *et al*. (2011) and Cheesbrough (2006). Aliquots from the last dilution were plated onto Nutrient Agar, Lactose Broth, MacConkey Agar, Eosin-Methylene Blue (EMB) Agar, Blood agar, Brain-Heart Infusion Agar and Mueller-Hinton Agar prepared according to manufacturer's instructions (Lammert, 2007). The plates were incubated aerobically at 37^{o}C for 24 or 48 hours, depending on the medium used. Formation of colonies and the physical appearances were observed and recorded. The morphological features (form, texture, consistency, shape, pigmentation, margin and elevation) of the obtained colonies was reported, and then pure cultures of the bacteria were subjected to Gram's stain and biochemical tests (catalase, citrate, indole, methyl red, Voges-Proskauer, motility and oxidase tests) according to the protocols of Cheesbrough (2006).

Molecular Characterization of Bacterial isolates

The identity of the isolated bacterial species was further confirmed through the amplification of the 16S rRNA gene of the members of the suspected bacterial genera using genus specific primers (Danbing *et al*., 1999).

DNA Extraction

The DNA sample for the experiments was extracted from pure colonies of the bacterial isolates which were inoculated into 3ml of Luria-Bertani (LB) broth medium, and incubated at 37^{o}C overnight. From the overnight culture, 1ml aliquot was centrifuged for 2 minutes at 16000 × g, and pelleted bacterial cells were re-suspended in 200µl of sterile distilled water thoroughly by vortexing, before being subjected to DNA extraction using Zymoresearch^{TM} Genomic DNA extraction kit (Irvin, CA. USA) following the manufacturer's instruction (Ganiyu, 2017).

DNA amplification and Electrophoresis of PCR amplicons

The identified bacterial isolates were subjected to polymerase chain reaction (PCR) for the detection and amplification of specific genes. The PCR reaction was carried out using commercially prepared primers (Olowe *et al*., 2017), and KAPATaq DNA polymerase. The primers used in the present study are (16S rRNA Bact 1442-F) sequence with 5'-AGA GTT GAT CCT GGC TCA G-3' and (16S rRNA Bact 1492-R) sequence with 5'-GGT TAC CTT ACG ACT T-3', as the forward and reverse primers, respectively (Hamid *et al*., 2020).

The total reaction volume was 25µL, with the reaction mix comprising of 2µL each of the genomic DNA, 2.5µL of 10 TaqA Buffer, 0.4M (0.85µL) each of forward and reverse primers, 1.25mM (1.5µL) of $MgCl_2$, 0.25mM (0.2µL) of dNTP mixed and 0.2µL of Taq DNA polymerase, in ddH_2O. Amplification was carried out by initial denaturation at 95^{o}C for 5 mins, followed by 35 cycles of denaturation at 94^{o}C for 30s, annealing at a 60^{o}C for 30s and extension at 72^{o}C for 1 min. This was followed by final extension at 72^{o}C for 10 mins. The PCR product was visualized on a 1.5% agarose gel stained with ethidium bromide (Olowe *et al*., 2017).

RESULTS

Conventional Isolation and Identification of Bacteria

On the basis of the conventional identification protocols of colonial morphology, Gram's stain and biochemical test reactions, 17 bacterial isolates were identified in this research. *E. coli* and *E. faecalis* (6/17, or 35.29%, each) were the most frequently identified isolates from the research, followed by *E. faecium* (5/17, or 29.41%) (Table 1).

Table 1: Conventional Identification of the Isolated Bacteria from Drinking Water Within Katsina Metropolis

Isolate No.	GS	Cat	Cit	Ind	L&M	Man	MR	Mot	TSIA	VP	Suspected Organism
SKWa	-	+	-	+	-	-	+	+	+	-	*E. coli*
SKWb	-	+	-	+	-	-	+	+	+	-	*E. coli*
SKWc	-	+	-	+	-	-	+	+	+	-	*E. coli*
SKWe	-	+	-	+	-	-	+	+	+	-	*E. coli*
SKWf	+	-	-	-	+	+	-	-	-	+	*E. faecalis*
SKWg	+	-	-	-	+	-	-	-	+	+	*E. faecium*
SKWi	+	-	-	-	+	-	-	-	+	+	*E. faecium*
SKRa	+	-	-	-	+	-	-	-	+	+	*E. faecium*
SKRh	-	+	-	+	-	-	+	+	+	-	*E. coli*
TDUh	+	-	-	-	+	+	-	-	-	+	*E. faecalis*
TKEc	+	-	-	-	+	+	-	-	-	+	*E. faecalis*

TKEd	+	-	-	-	+	-	-	-	+	+	*E. faecium*
TKEf	+	-	-	-	+	-	-	-	+	+	*E. faecium*
TKEh	+	-	-	-	+	+	-	-	-	+	*E. faecalis*
TKEj	-	+	-	+	-	-	+	+	+	-	*E. coli*
TKMa	+	-	-	-	+	+	-	-	-	+	*E. faecalis*
TKMi	+	-	-	-	+	+	-	-	-	+	*E. faecalis*

Legend:
GS = Gram's stain, Cat = Catalase, Cit = Citrate, Ind = Indole, L&M= Lactose & Maltose, Man = Mannitol, MR = Methyl red, Mot = Motility, TSIA = Triple sugar iron agar, VP = Voges proskeur, + = positive, - = negative.

Molecular Identification of the Bacterial Isolates
The 16S rRNA gene sequences of two bacteria were successfully amplified, as shown in the gel electrophoresis results (figure 1).

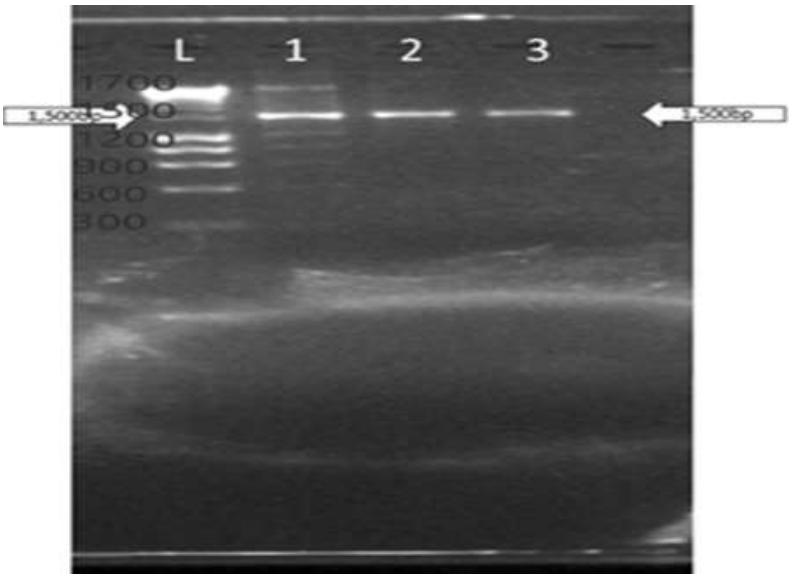


Figure 1: Agrose gel electrophoresis of the PCR amplicons from the 16S rRNA gene. The L lane represents the hyperladder (bioline, biosystems), 200bp to 10,000kb, while Lanes 1, 2 and 3 represent X, Y and Z samples.

After the sequences where obtained, they were individually identified through blasting using National Council for Biotechnology Information (NCBI)'s Basic Local Alignment Search Tool (BLAST). Nucleotide BLAST (NBLAST) was performed, where the sequences where pasted and analysed using the rRNA/ITS databases, for 16S rRNA sequences of bacteria and archaea. Uncultured samples were excluded from the search and the blasting was optimized for highly similar sequences (megablast).
The first bacterium was found to share the closest similarity to *Enterobacter kobei* strain CIP 105566 (97.86% similarity) and hence was assigned the name *Enterobacter kobei* AA01; while the second bacterium was found to share the highest similarity with *Escherichia marmotae* strain HT073016(79.53%) and hence was assigned the name *Escherichia marmotae* AN01.
Therefore, based on the results, the first bacterium belongs to the *Enterobacter kobei* species, due to the high % similarity (99.86%). However, the second bacterium has a sequence with a lower % similarity to the closest species of *Escherichia*, i.e. *Escherichia marmotae*, therefore,

the result predicts that the sequence may belong to an entirely novel bacterium (79.53%) (table 2).

Table 2: BLASTN sequence of *Enterobacter kobei* and *Escherichia marmotaae*

S/No	Scientific name of the bacterial specie	Max Score	Total Score	Query Cover	E value	% Identity	Accession Length	Accession Number
1	***Enterobacter kobei* strain AA01***	-	-	-	0.0	-	1035	-
2	*Enterobacter cloacae* subsp. *dissolvens* strain ATCC 23373	1291	1291	71%	0.0	97.99%	1449	NR_118011.1
3	*Enterobacterludwigii* strain EN-199	1291	1291	71%	0.0	97.99%	1513	NR_042349.1
4	*Enterobacterkobei* strain CIP 105566	1288	1288	71%	0.0	97.86%	1451	NR_028993.1
5	*Enterobacterbugandensis* strain 247BMC	1242	1242	70%	0.0	97.15%	1444	NR_148649.1
6	****Escherichia marmotae* AN01**	-	-	-	0.0	-	629	-
7	*Escherichia marmotae* strain HT073016	436	436	99%	0.0	79.53%	1504	NR_136472.1
8	*Escherichiacoli* strain NBRC 102203	442	442	99%	0.0	79.66%	1467	NR_114042.1
9	*Escherichia albertii* strain Albert 19982	422	422	93%	0.0	79.83%	1494	NR_025569.1
10	*Escherichiafergusonii* strain ATCC 35469	442	442	99%	0.0	79.69%	1542	NR_074902.1

Key: * = The sequence of the isolated bacterium (1), designated *Enterobacter kobei* strain AA01; ** = The sequence of the isolated bacterium (2), designated *Escherichia coli* AN01, - = Not applicable, E value = Error/random background noise.

The obtained results were used to generate a phylogenetic tree using Clustal Omega Online Software of the European Bioinformatics Institute, European Molecular Biology Laboratory, where ClustalW and Pearson/FASTA were chosen as the output format, and thereafter, a phylogenetic tree showing the cladogram was drawn using neighbor joining tree without distance corrections (figure 2 and 3).

Figure 3: Phylogenetic clustering of *Enterobacter* species nearest to the isolated organism's sequence. Enterobacter represents the bacterium's sequence (designated *Enterobacterkobei* AA01); kobei represents *Enterobacter kobei* strain CIP 105566, which shares the highest similarity with the sequence of the isolated bacterium; cloacae represents *Enterobacter cloacae* subsp. *dissolvens* strain ATCC 23373; ludwigii represents *Enterobacter ludwigii* strain EN-199 and bugandensis represents *Enterobacter bugandensis* strain 247BMC.

Figure 4: Phylogenetic clustering of *Escherichia* species nearest to the isolated organism's sequence. Escherichia represents the bacterium's sequence (designated *Escherichia coli* AN01); marmotae represents *Escherichia marmotae* strain HT073016, which shares the highest similarity with the sequence of the isolated bacterium; coli represents *Escherichia coli* strain NBRC 102203; albertiii represents *Escherichia albertii* strain Albert 19982 and fergusonii represents *Escherichia fergusonii* strain ATCC 35469.

DISCUSSION

The isolation of *Escherichia coli* and *Enterococcus faecalis* from the waters is in conformity with previous studies, such as Svagzdiene *et al*. (2010), where *Enterobacter kobei* was isolated from the samples, representing a novel water contaminant. The bacterium is grouped under the *Enterobacter cloacae* complex, and has been isolated from filtered water (Kamper *et al*., 2015). The predominant bacterium was *Escherichia coli*, and this is in agreement with previous findings of Ibemesim (2014) who isolated *Escherichia coli* from sachet and tap water in Abuja area.

Similarly, previous studies had confirmed the tendency of genetically related species to be isolated from similar water sources, e.g. *Escherichia coli* and *Enterococcus* species, such as *E. faecalis* and *E. faecium*, but from different localities; or, alternatively, the water samples of different localities may contain diverse bacteria as revealed by 16S rRNA gene sequencing, and this is typically due to environmental differences (Olowe *et al*., 2017). Enaigbe *et al*. (2019) reported the presence of bacteria in water storage tanks/reservoirs, and found out that the commonest bacteria belonged to the *Bacillus*, *Proteus* and *Pseudomonas* genera according to 16S rRNA sequence analysis. Previous studies (Oyeleke and Oduwole, 2009; Akpomie *et al*., 2012) have also reported the dominance of coliform bacteria in water. The capacity of some enteric bacteria to produce and secrete large quantities of extracellular enzymes (Schallmey *et al*., 2004), might have enabled them to dominate unsanitary water thus enhancing their growth and proliferation over other strains.

Previous studies had confirmed that 16S rRNA analysis is regarded as the gold standard in identification of microbes from water sources, and it is capable of revealing bacteria rarely identified in previous researches (Enaigbe *et al*., 2019; Olowe *et al*., 2017; Ghatak *et al*., 2013). Likewise, the bacteria identified in this study were rather new, and not stated much in previous studies. The first mention of *Enterobacter kobei* was by Kosako *et al*. (1996), where they described as Gram-negative, motile rods of the family *Enterobacteriaceae* isolate from clinical samples. These bacteria were also previously identified from water samples (river), and they pose a significant threat to public health, as they are associated with the production of carbapenamese enzyme, and hence antimicrobial resistance (Suzuki *et al*., 2020).

As for *Escherichia marmatae*, it was first described as a novel species in 2015 by Liu *et al.* (2015), as a Gram negative, non-sporulating, non-motile bacterium existing as short rods (0.5–161–2.5 mm), isolated from a rodent in the Himalayas, having close similarity with *Escherichia fergusonii* ATCC 35469T (99.3%), *Escherichia coli* ATCC 11775T (99.2%), *Escherichia albertii* LMG 20976T (98.9%), *Escherichia hermannii* CIP 103176T (98.4%), and *Escherichia vulneris* (97.7%). To the best of our knowledge, this study is amongst the first to report the presence of *Escherichia marmotae* as a potential faecal contaminant of water.

CONCLUSION

Using conventional methods of identification, this study identified *Escherichia coli*, *Enterobacter faecalis* and *Enterobacter faecium* as bacterial contaminants from drinking water samples within Katsina metropolis. However, based on 16S rRNA analyses, this study becomes among the first to report the presence of *Enterobacter kobei* and *Escherichia marmotae* as uncommon bacterial species that could contaminate sachet and tap water in the study area. It is recommended that regulatory agencies should encourage domestic and industrial sewage treatment in order to avoid point source contamination of sachet and tap water. Moreover, further studies are needed to definitively confirm the existence of these novel water contaminants from Katsina, using advanced techniques such as DNA-DNA hybridization and whole genome sequencing.

REFERENCES

Aderibigbe, S.A., Awoyemi, A. O. & Osagbemi, G.K. (2008). Availability, Adequacy and Quality of Water Supply in Ilorin Metropolis, Nigeria. *European Journal of Scientific Research, 23*, 528-536.

Akpomie, G. C., Ogbu, I. C., Osunkunle, A. A., Abuh, M. A. & Abonyi, M. N. (2012). Equilibrium isotherm studies on the sorption of lead from solution by Ehandiagu clay. *Journal of Emerging Trends in Engineering and Applied Sciences, 3*(2), 354-358.

Albertiri, H. (2018). Day by day they keep chipping away. *The Life Style Edit (TLSE), May 2018*, p. 7.

American Public Health Association – APHA (2017). *Standard methods for the examination of water and waste water* (23rd ed). American Public Health Association.

Ayotunde, E. O., Fagbenro, O. A. & Adebayo, O. T. (2011). Toxicity of aqueous extract of *Moringa oleifera* seed powder to Nile tilapia *Oreochromis niloticus* (LINNE 1779), fingerlings. *International Research Journal of Agricultural Sciences and Soil Science, 1*(4), 142-150.

Bernette, R. W. & Lancette, G. A. (2018). *Bacteriological Analysis Manual* (8th ed). AOAC International, pp. 140-142.

Cheesebrough, M. (2006). *District Laboratory Practice in Tropical Countries: Volumes I & II* (2nd ed)., Cambridge University Press.

Danbing, K. E., Francos, J., Picard, F. M., Roy, P. H., Ouellette, M. & Bergeron, M. G. (1999). Development of a PCR assay for rapid detection of *Enterococci*. *Journal of Clinical Microbiology, 37*(11), 3497-3503.

Enaigbe, A. A., Obhioze, A. A. & Stanley, I. O. (2019). Assessment of 16S rRNA sequence and heterotrophic plate count (hpc) methods of identifying bacteria from drinking water systems in Benin City Metropolis. *Bacterial Empire, 2*(3), 64-69.

Ganiyu, S. A., Yusuf, S. N., Agbolade, J. O. & Imonmium, J. E. (2017). The levels of yield and purity of genomic DNA from five tomato cultivars subjected to two DNA extraction techniques. *Nigerian Journal of Biotechnology, 33*(2017), 131-135.

Ghatak, S., Muthukumara, R. B. & Nachimuthu, S. K. (2013). A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. *Journal of Biomolecular Techniques, 24*(4), 224-31.

Hamid, M. E., Mahgoub, A., Babiker, A. J. O., Babiker, H. A. E., Holie, M. A. I., Elhassan, M. M. & Joseph, M. R. (2020). Isolation and identification of *Streptomyces* sp. from desert and savannah soils in Sudan. *International Journal of Environmental Research and Public Health, 17*, 8749.

Ibemesim, A. O. (2014). Comparative study of quality of sachet and bottle water sold on the street of Abuja, Nigeria.*South America Journal of Public Health, 2*(3), 311.

Jay, M. J., Loessner, J. M. & Golden, A. D. (2015). *Modern Food Microbiology* (7th ed.), Springer Science/ Business Media Inc., pp. 476-477.

Kamper, S. J., Maher, C. G. & Henschke, N. (2015). The epidemiological and economic consequences of pain. *Mayo Clinic Proceedings 90*(1), 139-147.

Kosako, Y., Tamura, K., Sakazaki, R. & Miki, K. (1996). *Enterobacter kobei* sp. nov., a new species of the family *Enterobacteriaceae* resembling *Enterobacter cloacae*. *Current Microbiology, 33*(4), 261-265.

Kumar, A. & Xagoraraki, I. (2010). Pharmaceutical, personal care products and endocrine-disrupting chemicals in US surface and finished drinking water: A proposed ranking system. *Science of the Total Environment 408*(23), 5972-5989.

Liu, S., Jin, D., Lan, R., Wang, Y., Meng, Q., Dai, H., Lu, S., Hu, S. & Xu, J. (2015). *Escherichia marmotae* sp. nov., isolated from faeces of *Marmota himalayana*. *International Journal of Systematic and Evolutionary Microbiology, 65*, 2130–2134.

Olowe, B. M., Oluyege, J. O., Famurewa, O., Ogunniran, A. O. & Adelegan, O. (2017). Molecular identification of *Escherichiacoli* and new emerging enteropathogen, *Escherichia fergusonii*, from drinking water sources in Ado-Ekiti, Ekiti State, Nigeria. *Journal of Microbiology Research*, *7*(3), 45-54.

Oyeleke, S. B. & Oduwole, A. A. (2009). Production of amylase by bacteria isolated from a cassava waste dumpsite in Minna, Niger State, Nigeria. *African Journal of Microbiology Research 3*(4), 143-146.

Schallmey, M., Singh, A. & Ward, O. P. (2004). Developments in the use of *Bacillus* species for industrial production. *Canadian Journal of Microbiology*, *50*(1), 1-17.

Svagzdiene, R., Lau, R. & Page, R. A. (2010). Microbiological quality of bottled water brands sold in retail outlet in New Zealand. *Water Science and Technology: Water Supply, 10*(5), 689-699.

WHO – World Health Organisation (2014). *Guidelines for drinking-water quality: fourth edition incorporating the first addendum*. World Health Organization. https://apps.who.int/iris/bitstream/handle/10665/254637/9789241549950-eng.pdf

Willey, J. M.., Sherwood, L. M. & Woolvertoon, C. J. (2011). *Prescott's Microbiology* (8th Ed.), McGraw-Hill Company, pp. 964- 968 & 1052-1064.

TOXICOLOGICAL EVALUATION OF AQUEOUS FRACTION OF *SOLANUM INCANUM* ROOT IN ALBINO RATS

[1]Abdullahi, N. S.
[2]Abdul, N.A*
[1]Abubakar, M.G.
[1]Saidu, Y.
[3]Bala A.Y
[1]Department of Biochemistry, Faculty of Sciences, Usmanu Danfodiyo University, Sokoto
[2]Department of Microbiology, Umaru Musa 'Yar'adua University, PMB 2218, Katsina, Nigeria
[3]Department of Biological Sciences, Faculty of Sciences, Usmanu Danfodiyo University, Sokoto

ABSTRACT
Solanum species are traditionally used in the treatment of several diseases, such as diabetes etc. It has been shown that plants contain many chemical compounds having medicinal values. These compounds are extracted from different parts of diverse plants such as root, stem, leaf, flower, fruit, etc. It is popularly believed that herbal plants are safer than pharmaceuticals because they are of natural origin. However, recent scientific reports have demonstrated that several medicinal plants used in herbal medicine are potentially toxic, and some are even mutagenic or carcinogen. Thus the present study was designed to investigate the toxicity effects of aqueous fraction of *Solanum incanum* root in albino rats. The OECD guidelines were used for the acute and sub-chronic studies of the fraction and the effect of the fraction on serum concentrations of liver and kidney function indices were determined. The result of the studies shows that the LD_{50} of aqueous fraction is greater than 5000mg/kg and its sub-chronic oral administration did not produce significant changes on the liver and renal indices. Hence, the finding suggested that the aqueous fraction of *Solanum incanum* root is scientifically safe and can be utilize for the management of disease conditions.
Keywords: *Solanum incanum,* Aqueous fraction, Toxicity, LD_{50} and sub-chronic

INTRODUCTION
Medicinal plants are plants used as natural medicines to treat and prevent illnesses (WHO, 2013). Plants have always been the most important source of drugs mainly because most plants are food producers and are able to synthesize a large variety of biochemical and organic compounds such as carbohydrates, proteins, terpenes, steroids, alkaloids and glycosides (Nweze *et al*., 2009). There are many chemical substances that have been synthesized from plants for drugs and medicines. However, many plants have also been reported to be unsafe to both animals and humans. It should therefore, be emphasized that the traditional use of any plant for medicinal activities, by no means, justify the safety of such plant (Ukwuani *et al*., 2012).
Plants in herbal medicine should therefore, be scrutinize for its safety or toxicity and necessary recommendations should be made on their use (Koduru *et al*., 2006). Acute and sub-chronic toxicity studies on medicinal plants and its products derived from them should be obtained in order to increase the confidence in its safety to humans, particularly for use in the development of active drugs (Ukwuani *et al*., 2012). Toxicity evaluations on medicinal plants

from them usually determine the level of safety particularly during the development of drugs (Jailoy *et al*., 2010).

Solanum incanum commonly called bitter or Sodom apple is one of about one thousand five hundred *Solanum* species in the world, it is well recognized medicinal plant belonging to the *Solanaceae* family (Schmelzer and Gurib-Fakim, 2008). It is commonly known as daata to the Hausas, anara to the Igbos and igbaa to the Yorubas. It is widely found in many parts of Africa, Middle East and Far Eastern Asia (Mwonjoria *et al*, 2014). Reported ethno-pharmacological activities of *Solanum incanum* include anti-microbial (Britto and Senthikumar, 2001; and Konate *et al*., 2011) anti-tumour (Chun-Nau *et al*., 2019), antinociceptive and antipyretic effects (Mwonjoria *et al*., 2011). *Solanum* species are also traditionally used in the treatment of diabetes (Nwanna *et al*., 2016). Okoli *et al*. (2009) reported the effect of *Solanum incanum* leaves on postprandial blood glucose concentration of normoglycemia Nigerians. The objective of this research is to evaluate the toxicity profiles of aqueous fraction of *Solanum incanum* root in albino rats.

MATERIALS AND METHODS

MATERIALS

Collection and Identification of Plant

Solanum incanum root was collected from VC's complex, Usmanu Danfodiyo University Sokoto and identified and authenticated by a Taxonomist from the Botany Unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria where a voucher specimen (UDUH/ANS/0242) was deposited.

Chemicals, Reagents and Equipment

All the chemicals and reagents used for this work were of analytical grade and purchased from reputable chemical manufacturers, e.g. Randox Laboratory, United Kingdom. The laboratory equipment used, were also of standard quality.

Animals Care

Twenty-five (25) healthy wistar strain albino rats of both sexes weighing between 130-150g were used for this study. The rats were purchased from the animal house of department of biochemistry, Usmanu Danfodiyo University, Sokoto. The rats were allowed to acclimatize to the environment and were maintained on standard laboratory diet and clean tap water and libitum for a period of one week. Animals were housed in clean cages under normal prevailing environmental condition. The protocol of the study was guided by the best International Guidelines in animal care (IRAC, 1983; NRC, 1997, NIH, 2008 and OECD, 2019).

METHODS

Preparation of Methanol Root Extract of *Solanum incanum*

The powdered root of *Solanum incanum* (500g) was soaked in 2.5 litres of methanol for 72 hours, after which it was filtered using a piece of clean, sterile, white Muslin cloth to remove debris and filtered on a whatman No.1 filter paper. The filtrate was concentrated using a rotatory evaporator at 450^{o}C. Subsequently the residual methanol was dried and the percentage yield was calculated (Saidu *et al*., 2016). The dried crude methanolic extracts were stored in plastic containers until required.

The fractionation of the methanol root extract of *Solanum incanum* was carried out according to modified method of Hassan *et al*., (2013). Fifty grams (50 g) of the methanol crude root extract was dissolved in distilled water and placed in a separating funnel. Equal volume of hexane was added and vortexed vigorously and allowed to separate into two layers (upper the hexane phase and the lower aqueous fraction). The Aqueous fraction was collected followed by the hexane phase. The Aqueous fraction was similarly treated with ethyl acetate, and butanol saturated with water. The fractions were labelled Hexane (HXN), Ethyl acetate (ETE), Butanol saturated with water (BSW) and the aqueous fraction (AF) respectively. The

fractions were concentrated using a rotatory evaporator at 45^{o}C and the residual organic solvent dried in a drying cabinet.

Acute Oral Toxicity Test of Aqueous Fraction of *Solanum incanum* Root (LD_{50} determination)

The acute oral toxicity testing was performed according to organization for economic and co-operation development 425 guidelines for testing of chemical (OECD, 2019). A total of five albino rats were randomly selected and used for the experiment. The animals were fasted overnight providing only water, after which aqueous fraction of *Solanum incanum* root was administered orally at a single oral limit dose level of 5000 mg/kg body weight. After the administration, food was withheld for further 3hrs. The animals were observed individually at least once during the first 30min after dosing, periodically at 8hrs, 14hrs, 24hrs, and 48hrs intervals (with special attention given during the first 4hrs). The observations include fatigue, loss of appetite, diarrhoea, salivation, grooming, respiratory distress and refusal to eat and drink. The animals were thereafter observed for a period of 14 days for any signs of delayed toxicity and mortality.

Sub-chronic Toxicity Study of Aqueous Fraction of *Solanum incanum* Root

Twenty albino rats were randomized into four (4) groups of five (5) rats each for sub-chronic toxicity study in which oral administration of different concentration based on the LD_{50} of the aqueous fraction of *Solanum incanum* root continues for 28 days.

1. Group-I: Normal Control: administered with Distilled water only.
2. Group-II: Receives 30% of the LD_{50}
3. Group-III: Receives 60% of the LD_{50}
4. Group-IV: Receives 80% of the LD_{50}

Sub-chronic oral toxicity test was performed according to the Organization of Economic Co-operation and Development guideline 407 for testing of chemicals (OECD, 2018).

At the end of the observation periods in the Sub-chronic toxicity tests, the animal was fasted overnight on the 28th and on 29th day, they were anaesthetized by dropping each of the animals successively in a transparent plastic jar saturated with chloroform vapor. The animals were then removed from the jar and blood samples collected from the animals through cardiac puncture. The blood samples were allowed to clot, centrifuged and serum collected was used for liver function and kidney parameters determination.

Marker of Toxicity

The following tests were conducted to investigate derangements in the liver and kidney function of animal used for the Sub-chronic toxicity studies.

Measurement of Aspartate Amino Transferase (AST)

Aspartate amino transferase catalytic activity was determined by the method of Reitman and Frankel (1957). Aspartate amino transferase activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. Exactly 0.5ml solution containing phosphate buffer (100 mmol/l, pH 7.4), L-aspartate (100 mmol/l) and α-oxoglutarate (2 mmol/l) incubated for 30minutes at 37^{o}C were added to 0.1ml of sample, followed by the addition of 0.5ml 2, 4-dinitrophenylhydrazine (2 mmol/l) that was incubated for 20 minutes at 25^{o}C, after which 0.4 mol/l NaOH was added. The contents were mixed and the absorbance was read against the blank after 5minutes at a wavelength of 546nm wavelength. The activity of AST in the serum was obtained from a standard curve.

Measurement of Alanine Amino Transferase (ALT)

Alanine amino transferase activity was determined by the method of Reitman and Frankel (2017). Alanine amino transferase is well measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine. Exactly 0.5ml solution containing phosphate buffer (100 mmol/l, pH 7.4), L-alanine (200 mmol/l) and α-oxoglutarate (2 mmol/l) incubated for 30minutes at 37^{o}C were added to 0.1ml of sample, followed by the

addition of 0.5ml 2, 4-dinitrophenylhydrazine (2 mmol/l) that was incubated for 20 minutes at 25^{o}C, after which 0.4 mol/l NaOH was added. The contents were mixed and the absorbance of each of the samples was taken against blank after 5 minutes at 546 nm wavelength. The activity of ALT in the serum was obtained from a standard curve.

Measurement of Alkaline phosphatase (ALP)

Alkaline phosphatase activity was estimated using the method of Sood (2019). Serum alkaline phosphatase hydrolyzes 4-nitrophenylphosphate to 4-nitrophenol and inorganic phosphate at alkaline pH (10.5). The intensity of the colour is directly proportional to the activity of ALP. The serum (0.02 ml) was placed into a labelled test tube and 1 ml of the alkaline phosphatase working reagent was added. The test tube content was mixed and the initial absorbance was read against air at 405 nm and at zero minute. A timer was started and absorbance was read at 405 nm after 1, 2 and 3 minutes.

Measurement of Bilirubin (Total and Direct)

This was determined by the calorimetric method of Jendrassik and Grof (2018). Direct bilirubin reacts with diazotized sulphanic acid in alkaline medium to form a blue coloured complex. Total bilirubin was evaluated in the presence of caffeine which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Total Bilirubin

Exactly 1.25ml solution containing sulphanilic acid (29 mmol/l), hydrochloric acid (0.17 N), sodium nitrate (38.5 mmol/l), caffeine (0.26 mol/l), sodium benzoate (0.52 mol/l) incubated for 10minutes at 20-25^{o}C were added to 0.2ml of sample, followed by the addition of 1.0ml solution containing Tartrate (0.93 mol/l) and sodium hydroxide (1.9 N). The solutions were mixed and allowed to stand for 5-30 minutes at 20-25^{0}C and absorbance of the sample at 578 nm was read against sample blank

Direct Bilirubin

A mixture amounting to about 2.25ml containing sulphanilic acid (29 mmol/l), hydrochloric acid (0.17 N), sodium nitrate (38.5 mmol/l), caffeine (0.26 mol/l), sodium benzoate (0.52 mol/l), Tartrate (0.93 mol/l) and sodium hydroxide (1.9 N) and 0.9 % NaCl were added to 0.2ml of sample. The solutions were mixed and allowed to stand for 10 minutes and read at 546 nm.

Measurement of Serum Albumin

Serum Albumin was determined using Bromocresol green binding method as modified by Doumas *et al*. (2017). Bromocresol green (BCG) reagent were added to 0.02ml of serum, 3g/dl standard protein (bovine serum albumin) and water for the test, standard and blank respectively. The tubes were mixed properly and the absorbance of the standard and test were read against the blank at 632nm.

Measurement of Total Protein

Total protein was estimated using the biuret reaction method by Tiez (2015). Into well labeled test tubes, appropriate volumes of the required solution were dispensed as follows: 0.02ml Distilled H_2O and 1.0ml Biuret, 0.02ml Standard and 1.0ml Biuret, 0.02ml Serum and 1.0ml Biuret. The contents were mixed and incubated at room temperature for 10 minutes and the absorbances were read at 540nm wavelength colorimetrically against reagent blank. Total protein Standard = 6.0 g/dl.

Measurement of Serum Creatinine

Serum creatinine concentration was estimated using Jaffe's reaction (Bartels and Bohmer, 1971). Into well labeled test tubes with appropriate volumes of the required solution of the test sample containing about 2.0ml of (Distilled water (ml), H_2SO_4 (ml), Sodium tungstate (ml) and Serum (ml)) and the standard solution also of 2.0ml contains mixture of (Distilled water (ml), H_2SO_4 (ml), Sodium tungstate (ml) and standard) were dispensed, thoroughly mixed and centrifuged for 10mins at 1500rpm. After which, the supernatant was collected and

further treated accordingly. This constitutes the second stage in serum creatinine estimation. The preparation was finally allowed to stand for 10mins then the absorbance read at 505nm.

Measurement of Serum Urea

Serum urea was determined using the Berthelot colorimetric method of Young (2017). Into carefully well labeled test tubes, appropriate volumes of the required solution, the test sample containing about 0.11ml of (0.01ml sample and 0.1ml Urease) and the standard solution also of 0.11ml containing a mixture of (0.01ml standard and 0.1ml Urease) were dispensed. The contents were mixed and then incubated at 37^0C for 10 minutes. This was followed by the addition of 2.5ml Phenol and 2.5ml Sodium hypochlorite. The solution was mixed immediately and incubated at 37^0C for 15 minutes. The absorbance of the sample and standard were read against the blank at 520 nm.

Measurement of Serum Sodium and Potassium Ions

Serum sodium and potassium ions were measured using flame photometry, (Cheesbrough, 2011). The flame photometer was calibrated using the standard solutions for sodium and potassium respectively. The meter was set at zero while aspirating distilled water of blank solution. It was again set at 100 % E (emission) reading while aspirating with the top concentration of the standards. The percentage emission readings were recorded for all the intermediate standard solutions. A standard curve on linear graph paper was plotted and the concentration of the elements in the sample solution was determined from the standard curve.

Measurement of Bicarbonate and Chloride Ions

Serum bicarbonate and chloride ions were measured using titration/volumetric method (Chapman, 2016).

Bicarbonate: To 50 ml of water, few drops of phenolphthalein, 0.25 % in 50 % alcohol were added. Sulphuric acid, standard 0.025 M was then titrated with the pink coloured solution produced drop wise every two or three seconds until the pink colour disappears. One or two drops of methyl orange were then added to the solution. The titration was continued to the methyl orange end point and the total reading was recorded.

Chloride Ions: Potassium chromate indicator (1ml) was added to the solution from the bicarbonate determination and titrated against silver nitrate. At end point, burette reading was recorded.

RESULTS

Acute Toxicity Oral Test of the Aqueous Fraction of *Solanum incanum*

Single oral administration of 5000mg/kg of last remaining aqueous fraction of *Solanum incanum* root in albino rats did not produce any visible sign or symptoms of toxicity or mortality in the treated animals. Behavioural changes such as grooming, loss of appetite, salivation, fatigue, diarrhoea as well as refusal to eat and drink were not observed over a period of 14 days. Therefore, the result indicated that the LD_{50} of the last remaining aqueous fraction of *Solanum incanum* root is greater than 5000mg/Kg.

Body Weight (g) Changes of Rats Administered Sub-Chronic Doses of Aqueous Fraction of *Solanum incanum*

The result of the body weight (g) changes of the rats administered sub-chronic doses of last remaining aqueous fraction of *Solanum incanum* is presented in Figure 1. When compared with the initial weight at week 0, rats in the control group had significant increase ($p< 0.05$) in body weight at week 3 and 4. Body weight of the rats administered with 1500 mg/kg, 3000 mg/kg and 4000 mg/kg body weight increased significantly ($p< 0.05$) at week 4 of sub-chronic doses administration.

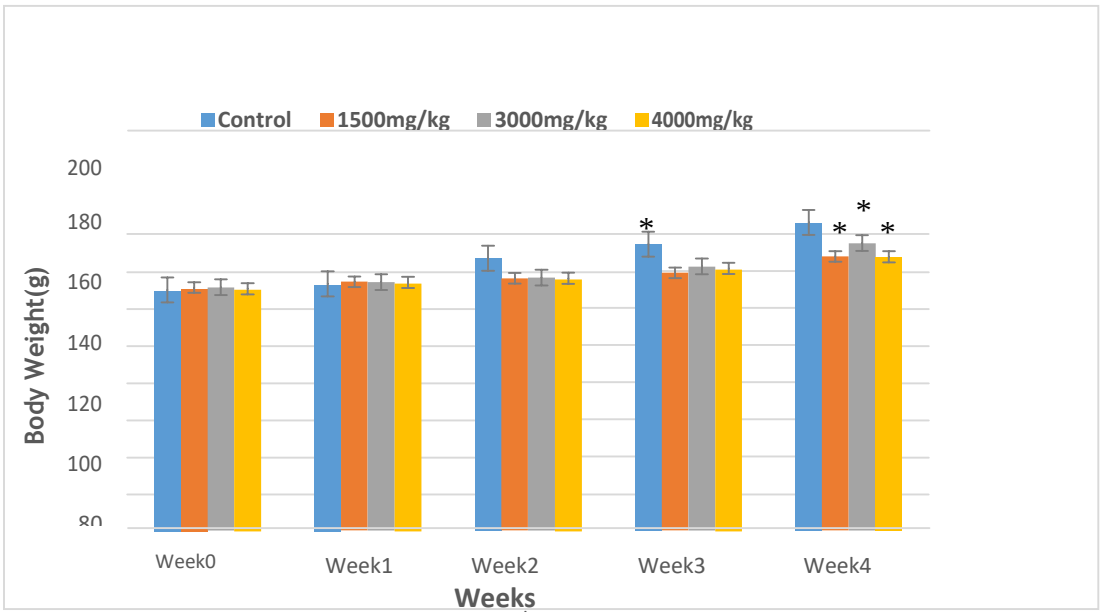


Figure1: Body Weight (g) Changes of the Rats Administered Sub-Chronic Doses of Last Remaining Aqueous Fraction of *Solanum incanum.* Values are mean ± SEM of 5 rats. *P< 0.05: significantly different when compared with initial weight

The Effect of Oral Administration of Aqueous Fraction of *Solanum incanum* on Liver Enzymes

The result of the liver enzymes activities of the rats administered sub-chronic oral doses is presented in Figure 2. There were no significant differences (p< 0.05) observed for the indices of liver enzymes, AST and ALT as compared with the control, but there was significant increase in ALP levels administered with 1500 mg/kg of oral doses of last aqueous remaining fraction of *Solanum incanum* as compared with the control.

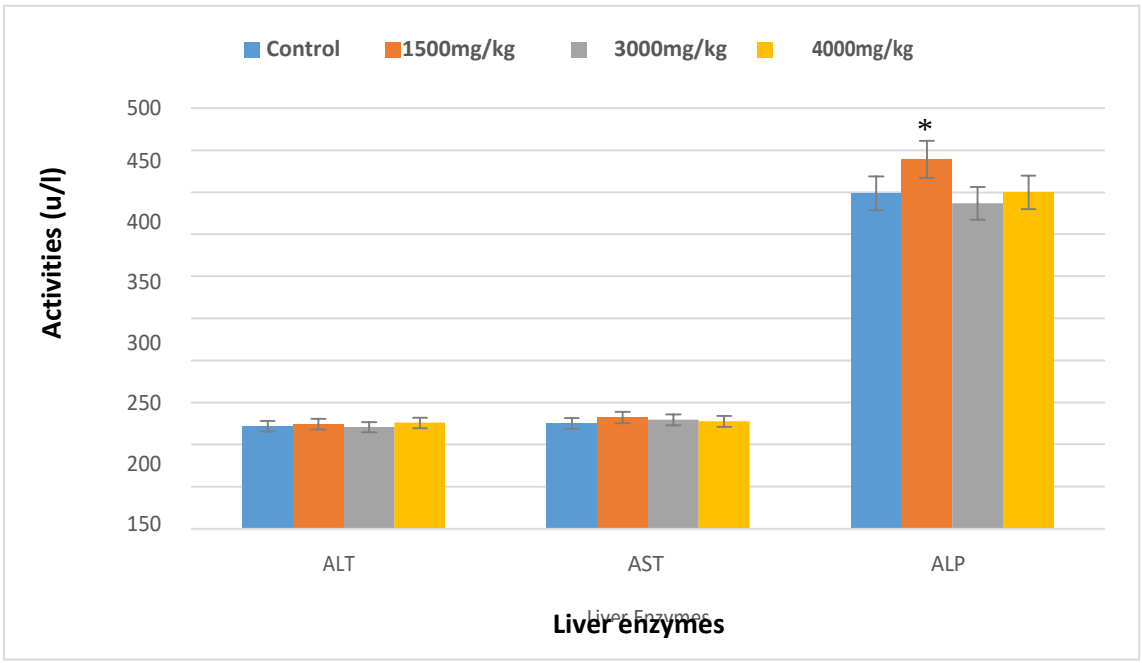


Figure 2: The Effect of Oral Administration of Aqueous Fraction of *Solanum incanum* on Liver Enzymes. AST-Aspartate Amino Transferase, ALT- Alanine Amino Transferase, ALP-Alkaline Phosphatase. Values are mean ± SEM of 5 rats. *P< 0.05: significantly different when compared with Control group

The Effect of Oral Administration of Aqueous Fraction of *Solanum incanum* on Serum Protein and Bilirubin Levels

The result of serum protein and bilirubin levels of rats administered sub-chronic doses of aqueous fraction of *Solanum incanum* is presented in Figure 4.3. There were no significant difference ($p<0.05$) observed for the level of serum protein and bilirubin in experimental rats administered with oral doses of aqueous fraction of *Solanum incanum* as compared with the control rats.

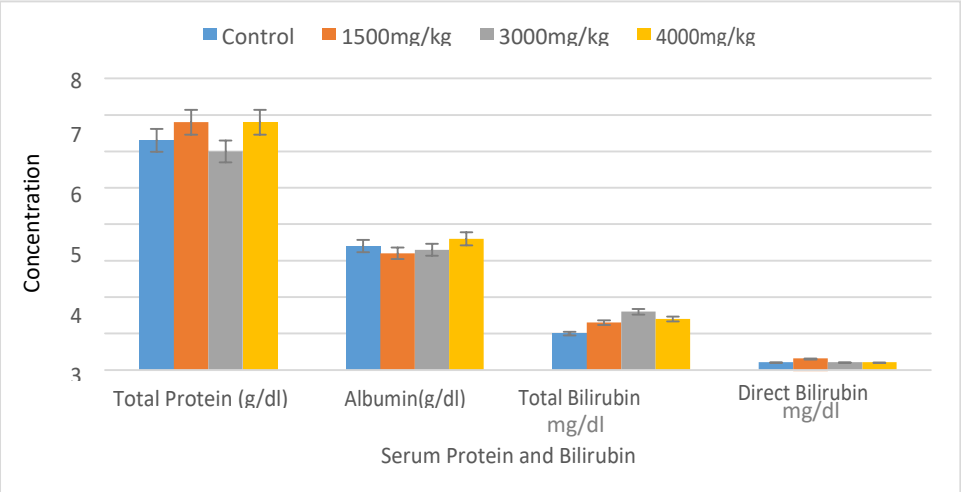


Figure 3: Serum Protein and serum Bilirubin Levels of the Rats Administered Sub-Chronic Oral Doses of Last Remaining Aqueous Fraction of *Solanum incanum*. Values are mean ± SEM of 5 rats. The values were not significantly different (P< 0.05) when compared with the control group.

The Effect of Oral Administration of Aqueous Fraction of *Solanum incanum* on Renal Function Parameters

The result of renal function parameters (Creatinine, urea, sodium, potassium, chloride and bicarbonate ion) is presented in Figure 4.4. There was mild elevation in creatinine levels among some of the aqueous fraction tested doses as compared with the control rats. Although, the elevation was not statistically significant at ($p<0.05$). There were no significant differences observed for the level of serum urea in the experimental rats administered with oral doses of aqueous fraction of *Solanum incanum* as compared with the control rats. Na^{+}, K^{+}, Cl^{-} , and HCO^{3-} levels of the aqueous fraction treated groups were not significantly different from those of the control group.

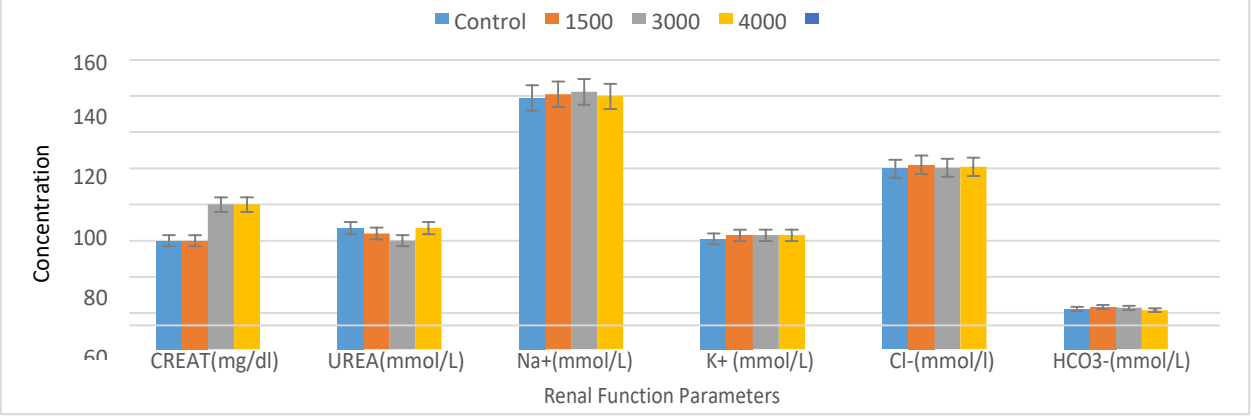


Figure 4: Renal Function Indices of the Rats Administered Sub-Chronic Oral Doses of Last Aqueous Remaining Fraction of *Solanum incanum*. Values are mean ± SEM of 5 rats. The values were not significantly different ($P< 0.05$) when compared with the control group. CREAT- Creatinine, Na^+- Sodium ion, K^+- Potassium ion, Cl^-- Chloride ion and HCO_3^-- Bicarbonate ion.

DISCUSSION

Determination of LD_{50} (Lethal dose that would kill 50% of the tested population) is usually the first step in the evaluation of toxic characteristic of a substance (Ogbuehi *et al*., 2015). According to Ukwuani *et al*. (2012), it is an initial appraisal of toxic manifestations and is one of the initial screening experiments performed with all compounds. The result of the acute oral toxicity of aqueous fraction of *Solanum incanum* was found to be greater than 5000 mg/kg body weight as there was no mortality and behavioral signs of toxicity, like grooming, loss of appetite, salivation, fatigue, diarrhoea, respiratory distress and refusal to eat and drink were not observed over a period of 14 days, in any of the experimental animals. This result is in line with previous work of Assefa *et al*. (2016) who reported that oral administration of aqueous root extract of *Solanum incanum* up to 15, 000 mg/kg doses of the extract to mice did not show signs of conventional toxicity. The Canadian Centre for occupational health and safety (CCOHS): based on the Hodge and Sterner Scale defines a test substance as extremely toxic when the LD_{50} is less than 1 mg/kg body weight, highly toxic at 1-50 mg/kg, moderately toxic at 50-500 mg/kg, slightly toxic at 500-5000 mg/kg, practically non-toxic at 5000-15000 mg/kg and relatively harmless when the LD_{50} is more than 15000 mg/kg (Hodge and Sterner, 2005).

The body weight of the animals administered sub-chronic doses of aqueous fraction of *Solanum incanum* increased at week four. The aqueous fraction may have improved the digestion and nutritive value of the animal's feed leading to efficient absorption and storage of excess macro-nutrients as reported by Ryosuke *et al.* (2012). This claim is further supported by the work of Sambo *et al*. (2010) who reported the presence of carbohydrate in *Solanum incanum* plant might be partially responsible for the observed increase in body weight of rats fed with various doses of the extract.

The expression of toxicity of xenobiotics is usually determined biochemically by monitoring some serum enzymes (Ukwuani *et al*., 2012). An increase in AST, ALT and ALP are commonly measured as indices of the liver damage (Abdel-Baset *et al*., 2017). Changes in ALT and AST activities may indicate alteration of cellular permeability or cellular injury and necrosis (Crook, 2016). Increased ALP level usually lead to cholestatic liver disease and biliary obstruction (Kaneko *et al*., 2017). ALT is more liver specific than AST and ALT activity is usually higher than AST activity at early or acute hepatocellular disease (Whitby *et al*., 1989). Although both of them are useful indices for identifying inflammation and death of the liver cells (Thapa and Walia, 2007). In this present study, the ALT and AST activities of

animals administered with oral doses of aqueous fraction of *Solanum incanum* were not significantly different ($P<0.05$) from the control but there was ALP elevation at 1500 mg/kg body weight of the extract, this does not signify liver damage because there was no increase in the level of ALT and AST whose elevation also signify liver damage.

Serum albumin and total proteins measurement are mostly performed to access the synthetic capacity of the liver (Thapa and Walia, 2017). Albumin is the most abundant plasma proteins with the functional role of maintenance of osmotic pressure, transportation of both endogenous and exogenous substances and serving as protein reserve (Saidu *et al*., 2017). Liver ability to produce albumin is lessened if the synthetic function of the organ is affected (Kulig and Vacanti, 2014). Rapid decrease in serum albumin is visible in hepatitis and liver cirrhosis (Saidu *et al*., 2017). Over hydration, impaired protein synthesis due to malnutrition, malabsorption, liver disease may result to decreased plasma total protein concentration (Yakubu *et al*., 2013). In the present study, the serum protein and albumin were not significantly different between the animals treated with sub-chronic doses and the control group. This result demonstrates the fact that the synthetic function of the liver of the rats exposed to various doses of aqueous fraction of *Solanum incanum* is not affected.

Bilirubin is a useful indicator of the excretory function of the liver, in addition to its being a useful device in the evaluation of haemolytic anaemia. In the present study, there was no significant different in total bilirubin and direct bilirubin in the serum of aqueous fraction of *Solanum incanum* rats when compared with the control. Therefore, it may be stated that the excretory function of the liver in rats is not affected significantly as a result of the sub-chronic oral administration of aqueous fraction of *Solanum incanum*.

Renal function parameters are usually determined to access the normal functioning of different parts of the kidneys (Abolaji *et al*., 2017). They are located in the liver and transported through the blood to the kidney for excretion (Oluwole *et al*., 2012). Functional kidneys remove these waste product compounds from the blood to be excreted in urine (Cotran *et al*., 2015). High level of these metabolites in the blood is an evidence of renal dysfunction. In this present study, there was mild elevation in the creatinine levels among some of the aqueous fraction tested doses (3000 and 4000 mg/kg body weight). Although, such elevation was not statistically significant ($p<0.05$) as compared with the control. There was no significant difference ($p<0.05$) in the urea levels of the animal treated group as compared with the control. This shows that aqueous fraction is not toxic to the kidney. Serum electrolytes such as sodium, potassium, bicarbonate and chloride play an important function in inter compartmental water balance and exchange of gases (Kang *et al*., 2012). In this present study, there was no significant difference ($p<0.05$) in the levels of sodium, potassium, chloride and bicarbonate treated groups as compared with the control group. This could be attributed to normal functioning status of the kidney.

CONCLUSION

The findings of the present study showed that aqueous fraction of *Solanum incanum* at the limit test dose is practically non-toxic and may be safe in oral formulation and could have greater importance as therapeutic agent in preventing or slowing diseases. Hence *Solanum incanum* is suggested as a potential plant that is scientifically safe and can be utilize for the management of disease conditions.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCE

WHO (2013). Research guidelines for evaluating the safety and efficacy of herbal medicines, Manila, *Journal of Plant Science*,**6**:4-12.

Nweze, N.E., Fakae, N.E. and Asuzu, I.U. (2009).Trypanocidal activity of the ethanolic extract of bucholziiCoriacea Seed, *Niger Veterinary Journal*, **29**:1-6

Ukwuani, A.N., Abubakar, M.G., Hassan, S.W. and Agaie, B.M. (2012). Toxicological studies of hydromethanolic leaves extract of *Grewiacrenata. International Journal of Pharmaceutical Sciences and Drug Research*, **4(4)**: 245-249.

Koduru, S., Grierson, D.S. and Afolayan, A.J. (2006). Antimicrobial activity of *Solanumaculeastrum*.*Pharmaceutical Biology*, **44**: 283-286.

Jailoy, K., Soonthornchareonnon, N., Lertprasertsuke, N., Panthong, A. and Sireeratawong, S. (2010). Acute and chronic oral toxicity of standardized water extract from the fruit of *phyllanthusemblicalinn. International Journal of Applied Research in Natural Product*, **3**(1):45-58

Schmelzer, G.H. and Gurib-fakim, A. (2008).Prota medicines plant foundation, Baclchleys publishers/ CTA waseningen, Netherlands, 525-528

Mwonjoria, J.K., Ngeranwa, J.J., Kariuki, H.N., Githinji, C.G. and Sagini, M.N. (2014). Ethno medicinal, phytochemical and pharmacological aspects of *Solanumincanum* (lin.). *International Journal Pharmacology and Toxicology*, **2**: 17-20.

Britto, S.J. and Senthinkumars, L. (2011). Antimicrobial activities of *Solanumincanum* leaf extract, *Asian Journal of Microbiology, Biotechnology and Environmental Science*, **3(1-2)**:65-66

Konate, K.M., Kiendrebeogo, M.B., Ouattara, A., Souza, A., Lamien-meda, Y. and Nongashida, N. (2011). Antibacterial potential acetone extracts from five medicinal plants used traditionally to treat infectious diseases in Burkina Faso. *Current Research Journal of Biological Sciences*, **3(5)**: 435-442

Chun-Nau, L., Chai-ming, L., Ming-kung, C., Kim-hong, G. and Sheng-jeng, W. (2019). The cytotoxic principles of *Solanumincanum*, *Journal of Natural Product*, **5** (3): 513-516

Mwonjoria, J.K., Ngeranwa, J.J., Kariuki, H.N., Githinji, C.G. and Sagini, M.N. (2014). Ethno medicinal, phytochemical and pharmacological aspects of *Solanumincanum* (lin.). *International Journal Pharmacology and Toxicology*, **2**: 17-20.

Nwanna, E.E., Emmanuel, O., Ibukun and Ganiyu O. (2016). Effect of some tropical egg plant fruits (*Solanumspp*) supplemented diet on diabetic neuropathy in male wistarratsinvivo. *Functional foods in Health and Disease*, 6 (10): 661-676.

Okoli, V.U., Okeke, E.C., Ehiemere, O.I. and Ezenduka O.P. (2009). Investigation of the effect of *Solanumincanum*on postprandial blood glucose concentration of normoglycemic Nigerians, *Pakistan Journal of Nutrition*, **8(10)**:1631-1635

IRAC (2013): The U.S Government Principles for the Utiliztion and Care for Vertebrate Animals Used in Testing, Researching and Training.

NIH. (2008): Guidelines for the Use of Non-Pharmaceutical-Grade Chemicals in Laboratory Animals. Animal Research Advisory Committee, Office of Animal Care and Use.

NRC. (2017): Occupational Health and Safety in the Care and Use of Research Animals. Washington: National Academy Press.

OECD. (2019): Guidance Document on Humane Endpoints for Experimental Animals Used in Safety Evaluation Studies. Paris: Organisation for Economic Co-operation and Development.

Saidu, Y., Suleiman, A.M., Abbas, A.Y., Onu, A., Tsado, I.A., Luba, M.L. (2016). Invitro screening for protein tyrosine phosphatase 1B and Dipeptidyl peptidase IV inhibitors

from selected Nigeria medicinal plants. *Journal of intercultural ethnopharmacology*, **2**:211-214.

Hassan, S.W., Verma, S., Srivastava, S.K., Luqman, S., Gupta, U. and Masood, N. (2013). Activity guided isolation and characterization of antioxidant and antibacterial agents from some local Nigerian plants. *African Journal of Biotech*nology, **12**(44):6315-6325.

OECD. (2011). Organization for economic co-operation and development. OECD guidelines for testing chemicals, acute up and down procedure no. **425**: 1-2

OECD. (2018). OECD guidelines for the testing of chemicals. Test No. 407: repeated dose 28-day oral toxicity study in rodents. Paris: *OECD Publishing*; **P**. 10.

Reitman, S. and Frankel, S. (2017); A calorimetric method for the determination of serum glutamic Oxaloacetic and glutamic pyruvic transminases.*America Journal of Clinical Pathology*, **28:**56-63.

Sood, R. (2019). Medical laboratory technology.Method and interpretation. Sed. Jaypeebrothers medical publishers Ltd., 488-490

Jendrassik, L. and Grof, P. (2018); Simplified photometric methods for the determination of bilirubin.Biochem.Zchr, **297**:81-89.

Doumas, B.T., Watson, W. and Briggs, H.G. (2011); Albumin standards and the measurement of serum albumin with bromocresol green. *Clinical Chemistry* Acda, **3**:187-196.

Tiez, N.W., (2015). Clinical guide to laboratory tests.3rd edition Philadelphia WB.Saunder, 268-273.

Bartels, H. and Bohmer, M. (2011). Micro-determination of creatinine.*Clinica ChimicaActa*., **32**:81-85.

Young, D.S. (2017). Effects of drugs on clinical laboratory test. *Annals of Clinical Biochemistry*.**34**:579-581.

Cheesbrough, M. (2011). Medical laboratory manual for tropical countries, Microbiology, ELBS, 2, **P**. 479.

Chapman, D.H. (2016); Methods of analysis for soils, plants and waters, university of Cal.

Ogbuehi, I.H., Ebong, O.O. and Obianime, A.W. (2015). Oral acute toxicity (LD_{50}) study of different solvent extracts of AbrusprecatoriusLinn leaves in Wistar rats. *European Journal of Experimental Biology*, **5(1)**:18-25.

Assefa, A., Urga, K. and Guta, A.(2016).Spasmolytic Activity of the Aqueous Root Extract of *Solanumincanum, Solanaceae*, *Ethiopian Journal of Biological Science* **(5)** 2.

Hodge, H.C. and Sterner, J.H. (2015). Determination of substances acute toxicity by LDB50B.*American Industrial hygiene Association*, **10**:93.

Ryosuke, F., Shinya, O., Yoko, N., Tin, N., Takumi, S., Satoshi, K. and Yasuo, K. (2012); Use of bean husk as an easily digestible fiber source for activating the fibrolytic rumen bacterium Fibrobactersuccinogenes and rice straw digestion, **83(10)**:696-703.

Sambo, H.S., Pam, C.S. and Dahiru, D. (2012). Effect of aqueous extract of *Solanumincanum* fruit on some serum biochemical parameters, *Agriculture, Business and technology Journal*, vol 10, **P** 82-86.

Abdel-Baset, H., El-Ahmady, O., Hassan, A.S., Abdel-Galil, F., Yosir, H. and Darwish, A. (2017). Biochemical effect of antioxidants on lipids and liver function in experimentally induced liver damage. *Annals of Clinical Biochemistry*, **34**: 656-663

Crook, M.A. (2006). Clinical chemistry and metabolic medicine. 7th ed. Hodder Arnold

Kaneko, J.J., Harvey, J.W. and Bruss, M.L. (1997).Clinical Biochemistry of domestic animals. 5th ed. Academic, USA, **P**. 285-302.

Whitby, L.G., Smith, A.F. and Becket, G.J. (2019). Lecture notes on clinical chemistry 9th ed. Blackwell scientific publications, oxford, London, Edinburgh, Boston, Melbourne, 20.

Thapa, B.R. and Walia, A. (2017). Liver function tests and their interpretation.*Indian Journal of Pediatric*, **74**:663-671.

Saidu, Y., Bilbis, L.S., Lawal, M., Isezuo, S.A., Hassan, S.W. and Abbas A.Y. (2017). Acute and sub-chronic toxicity studies of crude aqueous extract of *Albizziachevalieri* harms. *Asian Journal of Biochemistry*, **2(4)**: 224-236.

Kulig, K.M., and J.P. Vacanti. (2014). Hepatic tissue engineering, *Transplant Immunology*, 12 (3-4): 303-310.

Yakubu, M.T., Bilbis, L.S., Lawal, M. and Akanji, M.A. (2013). Effect of repeated administration of sildenafil citrate on selected enzyme activities of liver and kidney of male albino rate.*Nigeria journal of Pure and Applied Sciences*, **18**:1395–4000.

Abolaji, A.O., Adebayo, A.H. and Odesanmi, A. (2017). Effect of ethanolic extract of *Parinaripolyandra*(Rosaceae) on serum lipid profile andsome electrolytes in pregnant rabbits.*Research Journal of Medicinal Plants*.**1:**121- 127.

Oluwole, I.O., Folake, A.D. and Oluwaseyi, E.O. (2012); Sodium benzoate mediated hepatorenal toxicity in Wistar rat: Modulatory effects of*Azadirachtaindica*(Neem) leaf.*European Journal of Medicinal Plants*,**2(1)**:11-18.

Cotran, R.S., Kumar, V., Fausto, N., Robbins, S.L. and Abbas, A.K. (2005). Robbins and Cotran pathologic basis of disease.St. Louis, *Elsevier Saunders*, 72-87.

Kang, S., Kim, W. and Oh, M.S. (2002). Pathogenesis and treatment of hypernatremia.*Nephron*, **92(1)**: 14-17.

MOLECULAR DETECTION AND QUANTIFICATION OF CYTOMEGALOVIRUS DNA AMONG NEWBORN BABIES WITHIN KATSINA METROPOLIS

Nura, H.B.,[1]
Abdulkadir B.[1]
Abdullahi, S.[2]
[1]Department of Microbiology, Umaru Musa Yar'adua University, Katsina, Katsina State
[2]Department of Clinical Pharmacology, Faculty of Medicine, Umaru Musa Yar'adua University, Katsina, Katsina State

ABSTRACT
Cytomegalovirus (CMV) infection during pregnancy is a major cause of congenital infection worldwide. It can result in significant morbidity, mortality, or long-term sequelae, including sensorineural hearing loss. This study aimed to detect and quantify CMV among newborn babies in Katsina Metropolis by evaluating the prevalence, viral load and some associated risk factors that increases the susceptibility of newborns to the virus. Hundred (100) saliva samples were collected from newborns (aged between 1-12days) from selected hospitals and healthcare centers. Molecular analysis was carried out for the detection of the presence of HCMV DNA in the samples through DNA extraction and PCR amplification. And the viral load was determined. The results for the PCR showed that 6 out of 100 (6%) of the newborns were found to be HCMV positive while 94 out of 100 (94%) were found HCMV negative. The viral load reveals a load of 1.68×10^2, 6.32×10^3, 3.63×10^3, 1.41×10^3, 1.00×10^2 and 9.02×10^3 IU/ml respectively. The data shows 6% prevalence of CMV infection and the feasibility of CMV screening by testing qPCR. There was a weak relationship between maternal and neonatal CMV infection. There is no statistical association of age, birthweight, gender, delivery mode and gestational age and CMV DNA positivity. ($P>0.05$). This study demonstrated that PCR is a reliable test in the diagnosis of cytomegalovirus as it detects and quantifies viral DNA which is useful in predicting the patient's risk for disease and monitoring the effect of antiviral therapy. It is recommended that newborns with a positive PCR need to be vigilantly followed up for development of symptoms and early initiation of therapy.
Keywords: Cytomegalovirus, congenital, PCR.

INTRODUCTION
Human cytomegalovirus (CMV) is a prototype species member of Betaherpesvirinae, a subfamily of the Herpesviridae family which is highly prevalent in the human population, with seroprevalence ranging from 45% to 100% (Putri *et al*., 2019). CMV is endemic worldwide in the human population without seasonal variation (Alwan *et al*., 2019). Human cytomegalovirus infection is the most frequent cause of congenital infection worldwide and is the leading cause of permanent disability and birth defects (Alwan *et al*., 2019). Cytomegalovirus is the also cause of mental retardation, congenital abnormalities, and neonatal infections (Yasir *et al*., 2020). CMV is mainly a problem for certain high risk groups, these are unborn babies whose mothers become infected with CMV during the pregnancy and children or adults whose immune systems have been weakened by disease or drug treatment, such as organ transplant recipients or people infected with HIV. The symptoms of a CMV infection vary depending upon the age and health of the person who is infected, and how the infection occurred. Infants who are infected before birth usually show no symptoms of a CMV infection after they are born, although some can develop hearing, vision, neurological,

and developmental problems over time. In a few cases, there are symptoms at birth, which can include premature delivery, being small for gestational age, jaundice, enlarged liver and spleen, microcephaly (small head), seizures, rash, and feeding difficulties (Musa, 2014).
Identification of newborns with CMV infection is desirable because early interventions, including antiviral therapy for selected infants and identification of hearing loss that develops during early childhood, have been shown to result in better hearing and developmental outcomes (Goldfarb *et al*., 2020). However, most newborns with CMV infection are asymptomatic or have mild, non-specific findings, such that only a small fraction of infections are identified in the absence of coordinated neonatal CMV testing programs (Goldfarb *et al*., 2020). Cytomegalovirus (CMV) is a major public health problem throughout the world. It has been considered the most common infectious cause for intellectual disability. It is also the most common non-genetic cause of neurological disabilities in children that result to severe sequelae such as sensor neural hearing loss, neurodevelopmental delay and blindness (Ahmad *et al*., 2011). Most infants with CMV infection lack any clinical abnormalities and are not identified in the newborn nurseries (Mina *et al*., 2017). Moreover, there has not been any concise statistics regarding the prevalence of this disease in Katsina State, specifically in Katsina Metropolis.

MATERIALS AND METHODS

Ethical Considerations

Ethical clearance was obtained from Research and Ethical Clearance Committee Katsina State Ministry of Health (MOH/ADM/SUD/1152/1/353) and Federal Medical Centre (FMCNHREC.REG.N003/082012) Respectively. Respect to subject's rights was observed, this includes the right to refusal. There was provision for subject's safety (physical and psychological) by maintaining confidentiality.

Study Design

This is a hospital based cross-sectional study with both descriptive and analytical components. The descriptive component describes the occurrence of CMV among newborn babies in terms of person, place and time while the analytical component identifies the factors associated with CMV infection.

Sampling Size Determination

A total of 100 newborn babies were recruited into the study. The sample size was obtained using the formula as follows:

$$N = \frac{Z^2 (P) Q}{D^2}$$

Where,
N= Sample size
Z= Statistics for a level of 95% confidence interval = 1.96
P= Prevalence rate of CMV previous studies = 3.8%
D = level of significance (allowable error) = 5% or 0.05
Q= 1-P

$$N = \frac{Z^2 (P) Q}{D^2}$$

$$N = \frac{(1.96^2) \times 0.038 \times (1-0.038)}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.038 \times 0.962}{0.0025}$$

$$=56.17$$

Based on the calculated sample size above, a sample of 56.17 was the least sample size for the study. However, 30-40% of the calculated sample size was added to the 56.17 to avoid error. Therefore, an approximate total of 100 consecutive non duplicate samples were collected and used for the study.

Sample Collection

Saliva sample was taken from neonates using a Dacron swab, where the swab was placed on neonates' buccal mucosa until moist and the specimen was then placed into a viral transport medium, then transferred to the research center as soon as possible. Data from routine clinical evaluations of the newborns, including gestational age, gender, weight, prematurity, congenital anomalies, and concomitant diseases, were collected from the medical record. Data of the mothers including demographic and clinical information as well as maternal history of pregnancy were obtained from interviews conducted by research staff and from medical records.

DNA Extraction

The samples were rapidly shaken, and the resulting liquid was stored at -20 °C until the testing time. In the laboratory, DNA samples were extracted using a DNA extraction kit using the Liferiver (Nucleic Acid Extraction Kit) in accordance with the manufacturer's instructions as follows brief:

20µl of proteinase K and 600µl of Lysis Buffer were added to a 2ml microcentrifuge tube, 300µl of each sample was then added and immediately mixed thoroughly by vortexing for 15s prior to incubation at 56°C for 10min. The tube was then centrifuged briefly to remove drops from inside the lid, and 600µl of molecular biology grade 100% ethanol added to the sample, mixed by vortexing for 15s, followed by brief centrifugation to remove drops from inside the lid. Binding of sample to the Mini spin column membrane was achieved in two successive steps, each involving the application of all the sample mixture to a Mini spin column in a 2ml collection tube followed by centrifugation at 8000rpm for 1min. The filtrate with collection tube was discarded and spin column placed in another clean 2ml collection tube. Wash steps involved addition of 600µl of Buffer A, centrifugation at 8000rpm for 1min, followed by 600µl of Buffer V and centrifugation at 12,000rpm for 3min, with change of collection tube at the end of each centrifugation step. Additional centrifugation was performed at 12,000rpm for 1min before the elution step. DNA was eluted into 50µl Elution added to the spin column placed in a sterile 1.5ml microcentrifuge tube. Following incubation at room temperature for 5min, final centrifugation at 8000rpm for 1min was performed to recover the DNA. The extracted DNA samples were stored at -20ºC prior to analysis.

PCR Amplification

DNA copy numbers were computed in a TaqMan Real-Time assay run on the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems® Inc.). Each qPCR reaction was in a 36µl volume consisting of 34.6µl HCMV PCR Reacion Buffer (Bioer Technology), 1µl internal control, 0.1µl UDG, 0.3µl Taq polymerase and 4µl template DNA. All standards and samples were run in duplicate. Cycling conditions were 37°C for 5min, 94°C for 1min, followed by 40 cycles of 94°C for 5s and 60°C for 40s. FAM and HEX channels were chosen for collecting signals.

Statistical Analysis

All data were entered into restricted, password-protected Microsoft Access 2016 databases, and related hard copy information kept in a locked cabinet. SPSS and Microsoft Excel 2016 was used for data analysis. Nominal data were analyzed in contingency tables by Fisher Exact tests, while sample means were compared by the Student's t test and medians analyzed by the Mann-Whitney U test. P-values were computed with significance threshold set at 0.05.

RESULTS

A total of 100 saliva samples of newborn babies at the five selected Hospitals and Healthcare centers in Katsina Metropolis were randomly collected. Samples were collected from General Hospital Katsina (20), Turai Maternity and Children Hospital Katsina (20), Federal Medical Centre Katsina (20), Comprehensive Healthcare Centre Kofar Kaura (17), Comprehensive Healthcare Centre Kofar Marusa (13), Sahel Medicare Centre Katsina (10) and analyzed. Out of 100 samples tested 6 (6%) emerged positive and 95 (95%) emerged negative. Thus, the prevalence of cytomegalovirus among newborn babies within the study area was 6% (Table 1). The viral load of infected newborns and their mothers were quantified. The mothers have the highest load of 10^4 and 10^3 copies/mL with the newborn babies having a load of 10^3 and 10^2 copies/mL. The mean viral load in newborn babies was 3441 copies/mL and 8930 copies/mL in their respective mothers (Table 2).

No statistically significant association between age (days) and CMV infection (P=0.06), hence all newborns have the same possibility of becoming infected. Similarly, the gestational age, birthweight of the newborn babies as well as the gender of the newborn babies are all not significantly associated with CMV infection statistically, in this study (P>0.05). Although the proportion of newborn babies with who were delivered vaginally (4.95%) was higher than the proportion of newborn babies with CMV infection delivered by Caesarean Section (0.99%) this difference is not statistically significant (X^2=0.10, df=2, P=0.009). However, neonates who were delivered vaginally were about 4 times more likely to have CMV infection compared to those delivered by Caesarean Section (Table 3). There is a positive but weak relationship between maternal and neonatal CMV infection. However, this relationship is not statistically significant (r=0.37, p=0.945). Pearson's correlation analysis was performed to assess the correlation between the quantitation of CMV DNA in newborn babies and their mothers (Table 4).

Table 1: Prevalence of CMV infection of the study subjects

CASES	N(%)
Positive	6 (6)
Negative	95 (95)
Total	100(100)

Table 2: CMV viral load of positive newborns and their mothers

Maternal (copies/mL)	Neonatal (copies/mL)
4.45×10^3	1.68×10^2
1.95×10^3	6.32×10^3
1.30×10^4	3.63×10^3
2.20×10^4	1.41×10^3
1.28×10^3	1.00×10^2
1.09×10^4	9.02×10^3

Table 3: Sociodemographic factors/Risk factors associated with CMV infection

VARIABLE		CMV INFECTION				χ^2	df	P-value
		POSITIVE		NEGATIVE				
		N	%	N	%			
AGE (DAYS)	1-4	3	3	26	26			
	5-8	3	3	29	29	0.22	3	0.06
	8-12	0	0	39	39			
BIRTH WEIGHT	<1.5kg	0	0	12	12			
	1.5-2.5kg	0	0	23	23	0.002	3	0.08
	>2.5kg	6	6	59	59			
GENDER	Male	5	5	47	47	0.10	2	0.05
	Female	1	1	47	47			
DELIVERY MODE	Vaginal	5	5	27	27	0.10	2	0.09
	CS	1	1	67	67			
GESTATIONAL AGE	Term	6	6	84	84	0.01	2	0.15
	Preterm	0	0	10	10			

Table 4: Co-relation between CMV infected newborns and their mothers

Maternal (copies/ml)	Neonatal (copies/ml)	R	P
4450	168	0.37	0.945
1950	6320		
13000	3630		
22000	1410		
1280	100		
10900	9020		

DISCUSSION

Compared to the world investigations, CMV in newborns are scarce in Africa. The number of neonates tested for CMV infection varied from 115 to 2685, and all clinical specimens were collected within 2 weeks of birth. In 9 studies PCR techniques were used for the detection of CMV, urine culture in 2, and antibody detection with ELISA in a single study. Most of the studies have used urine or saliva samples while only one study has used umbilical cord and nasopharyngeal aspirates.

Based on the available published literature, our study is the first to provide a report on CMV infection from a prospective screening study in Katsina. In our study, viral DNA was detected in the saliva of 6 out of 100 newborn babies, yielding a prevalence rate of CMV infection of 6% in newborn babies in Katsina. Thus, by using a sensitive molecular assay to detect CMV DNA in newborns' saliva samples, we found that one in every eighteen newborns in our study population was infected with CMV.

The 6% prevalence of CMV infection found in our study is higher compared to other newborn screening studies in Finland (0.2%) (Puhakka *et al*., 2018), Japan (0.3%) (Yamaguchi *et al*., 2017), Iran (0.5%) (Karimian *et al*., 2016), Panama (0.6%) (Estripeaut *et al*., 2007), China (0.7%) (Wang *et al*., 2017), Brazil (1.1%) (Mussi-Pinhata *et al*., 2009), India (2.1%) (Dar *et al*., 2008), Lagos, Nigeria (3.8%) (Olusanya *et al*., 2016), and Ethiopia (4.1%) (Kiros *et al*., 2021) but closely related to those observed in a peri-urban birth cohort in Gambia (5.4%) (Van der Sande *et al*., 2007) and in a highrisk population in China (6.1%) (Zhang *et al*.,

2007). The maternal CMV immune status was assessed in our study to correlate between maternal and neonatal CMV status which shows a weak positive relationship between them. However, our findings have a lower prevalence than that reported in Mozambique (6.3%) (Hailemariam *et al*., 2021).

Nevertheless, this study explored the use of a virologic assay (PCR) that is reliable and convenient to identify CMV-infected newborns. Although CMV seroprevalence was not determined as part of this study. A previous study conducted by Adeiza *et al* in Zaria, Nigeria detected CMV IgG in 86% and CMV IgM in 13.2% in HIV positive patients (Adeiza *et al*., 2016). Similarly, a previous study among pregnant women in Lagos, Nigeria showed that 97.2% were CMV-IgG positive (Akinbami *et al*., 2011). High prevalence (2.9%, 22/748) of CMV in HIV-exposed infants was also documented in a recent study from South Africa (Manicklal *et al*., 2014). However, this study demonstrates no statistically significant association between CMV infection and age, birthweight, gender, delivery mode and gestational age. This is similar to the previous findings in Lagos, Nigeria that indicated no associations between CMV and maternal age, mode of delivery, gestational age, birth weight, gender and jaundice (Olusanya *et al*., 2016).

Moreover, out of 6 cases positive for CMV DNA, the cases were having a viral load of 1.68×10^2, 6.32×10^3, 3.63×10^3, 1.41×10^3, 1.00×10^2 and 9.02×10^3 IU/ml respectively. According to study by Kraft *et al*., most Laboratory Developed Tests using Real-time polymerase chain reaction technologies, the linear range of CMV viral load tests is usually at least 6 log10 ($1x10^6$ IU/ml). A review of CMV viral load testing performed in 2009 at Emory Medical Laboratories showed 7.9% samples were positive for CMV DNA. Of these 58.7% were 100 000 copies/mL ($1x10^5$ IU/ml). [One IU/mL of CMV DNA is approximately 1.72 copies/ml]. Although low viral load values are common, their clinical significance is unclear. Very high viral load values are uncommon but when they do occur, are usually seen in immunosuppressed patients with primary CMV infection or in those with very severe disease. Because viral load values as high as 10 million ($1x10^7$ IU/ ml) may be present in some individuals, the upper limit of quantification of a test should be in this range. Some patients have persistent low levels of CMV DNA in plasma or whole blood and never develop CMV disease or relapse. In these individuals, it is more useful to follow trends in viral loads values over time than to assess the significance of any given viral load value.

In another prospective study by Forner *et al*., (2014) a CMV DNA viral load > 12,000 copies/ml (10,920 IU/ml or $1x10^4$ IU/ml) at birth was associated with an increased risk of late-onset CMV disease in infants with asymptomatic congenital CMV infection. In the same population, a birth CMV DNA viral load > 17, 000 copies/mL (15,470 IU/mL or $1.5x\ 10^4$ IU/ml) was associated with an increased risk of sensorineural hearing loss (Forner *et al*., 2015). Hsiao *et al* suggested CMV DNA viral load threshold of 11,830 IU/ml for the initiation of pre-emptive therapy in infants with suspected CMV pneumonia (Hsiao *et al*., 2013). Mayaphi *et al* suggested CMV DNA viral load threshold of 910 IU/ml for the initiation of preemptive therapy in HIV-positive patients (CD4 < 200 cells/ μL) in ICU (Mayaphi *et al*., 2014). Martin-Gandul *et al* established a viral load of 3983 ($3.9x\ 10^3$ IU/ml) as the optimal cut-off for initiating preemptive therapy in solid organ transplants.

Studies revealed that PCR assay of saliva showed high sensitivity and specificity and identifies congenitally infected newborns that would be missed by rapid culture (Hailemariam *et al*., 2021). Serologic CMV IgG and IgM assays are not suggested for the diagnosis of CMV infection in newborns because only 20%-70% of infected babies will have a positive CMV IgM antibody titer and many newborns will have a positive CMV IgG antibody titer from blood passed to them from their mother (Hailemariam *et al*., 2021).

CONCLUSION

The present study was conducted with the objective to scrutinize the prevalence of CMV infection, so that the prevention, prognosis and management can be made easy. Overall, the prevalence rate of CMV in the study area is higher compared to the previous findings. However, it shows a week association between maternal and neonatal CMV infection within the study area, unlike the association between risk factors and CMV infection which shows no relationship. Furthermore, CMV DNA viral load of both the newborns and the mothers was low and thus the clinical significance is unclear. Furthermore, patients with a positive PCR need to be vigilantly followed up for development of symptoms and early initiation of therapy.

Author contributions

NHB, AB and AS designed the experiments. NHB conducted the experiments. NHB wrote the manuscript, and AB and AS reviewed the manuscript.

Funding statement

This research received no external funding.

Disclosure statement

No potential conflict of interest was reported by the authors.

REFERENCES

Nina Dwi Putri, Ageng Wiyatno, Rama Dhenna, IdaYusSriyani, Aghnianditya Kresno Dewantari, Setyo Handryastuti (2019). Birth prevalence and characteristics of congenital cytomegalovirus infection in an urban birth cohort, Jakarta, Indonesia. International Journal of Infectious Diseases. 86:31–39

Alwan N. Sevan, Haidar A. Shamran, Avan H. Ghaib, Haider S. Kadhim, Qasim S. Al-Mayah, Atheer J. AL-Saffar, Ali H. Bayati, Hala S. Arif, Jianmin Fu and Brian L. Wickes (2019). Genotyping of Cytomegalovirus from Symptomatic Infected Neonates in Iraq. American Journal of Tropical Medecine and Hygiene 100(4) : 957–963. doi:10.4269/ajtmh.18-0152

Saif Jabbar Yasir, Younis Abdul-Redha AL- Khafaji, Khalida K Abbas Al-Kelaby (2020). Cytomegalovirus UL83 Gene as Diagnostic Marker for Preliminary Infection in Pregnant Women in AL-Najaf City. Systematic Review of Pharmacy. 2020; 11(5): 123 129

Musa Kalamullah Bawa (2014). The Prevalence and Associated Factors For Cytomegalovirus Infection Among Blood Donors In Minna, North Central Nigeria (Thesis). Ahmadu Bello University, Zaria, Nigeria.

Gantt S, Goldfarb DM, Park A, Rawlinson W, Boppana SB, Lazzarotto T, Mertz LM (2020). Performance of the Alethia CMV assay for detection of cytomegalovirus by use of neonatal saliva swabs. Journal of Clinical Microbiology 58:01951-19. https://doi.org/10.1128/JCM.01951-19

Ahmad, RM, Kawo, AH, Udeani, TKC (2011). Seroprevalence of Cytomegalovirus antibodies in pregnant women attending two selected hospitals in Sokoto state, Northern Nigeria. Bayero Journal of Pure and Applied Science 4(1):63-66

Mina Ebrahimi-Rad, Talayeh Seyed Shakeri, Fariba Shirvani, Kiana Shahrokhi and Nader Shahrokhi (2017). Prevalence of congenital cytomegalovirus infection in symptomatic newborns under 3 weeks in Tehran, Iran. BMC Infectious Diseases.17:688

Puhakka, Laura (2019). Congenital Cytomegalovirus Infection in Finland (Dissertation). University of Helsinki, Finland

Wang C, Zhang X, Bialek S, Cannon MJ (2011). Attribution of congenital cytomegalovirus infection to primary versus non-primary maternal infection. Clinical and Infectious Diseases; 52: e11-3.

Yamaguchi A, Ohishi T, Arai T (2017). Screening for seemingly healthy newborns with congenital cytomegalovirus infection by quantitative real-time polymerase chain reaction using newborn urine: an observational study. BMJ.7:013810. doi:10.1136/bmjopen-2016- 013810

Estripeaut D, Moreno Y, Ahumada Ruiz S, Martínez A, Racine JD, Sáez-Llorens X (2007). Seroprevalencia de la infección por citomegalovirus en puérperas y su impacto neonatal [Seroprevalence of cytomegalovirus infection in puerperal women and its impact on their newborns]. An Pediatric (Barc). 2007 Feb;66(2):135-9. Spanish. doi: 10.1157/13098930. PMID: 17306099.

Mussi-Pinhata MM, Yamamoto AY, Moura Brito RM, de Lima Isaac M, de Carvalho e Oliveira PF, Boppana S, Britt WJ (2009). Birth prevalence and natural history of congenital cytomegalovirus infection in a highly seroimmune population. Clinical and Infectious Diseases. 15;49(4):522-8. doi: 10.1086/600882. PMID: 19583520; PMCID: PMC2778219

Kiros M, Geteneh A, Andualem H, Alemu D, Tesfaye A, Tefera DA, *et al*. (2021) Human Cytomegalovirus infection among treatment-naïve HIV-1 infected patients in Ethiopia. PLoS ONE 16(2): e0247264. https://doi.org/10.1371/journal

Hailemariam M, Mekonnen Z, Claeys G, Padalko E (2021) Congenital Cytomegalovirus Infections: (no) Focus on Africa: A Review Gynecology and Obstetics (Sunnyvale) 11:545.

Adeiza MA, Dalhat MM, Musa BOP, Muktar HM, Garko SB, Habib AG (2016). Seroepidemiology of Cytomegalovirus Antibodies in HIV-positive and HIV-negative Adults in Nigeria. Sub-Saharan African Journal of Medicine. 3:142-7

Akinbami AA, Rabiu KA, Adewunmi AA, Wright KO, Dosunmu AO, Adeyemo TA, Adediran A, Osunkalu VO (2011). Seroprevalence of cytomegalovirus antibodies amongst normal pregnant women in Nigeria. International Journal of Womens Health. 3:423–8.

Manicklal S, van Niekerk AM, Kroon SM, Hutto C, Novak Z, Pati SK, Chowdhury N, Hsiao NY, Boppana SB (2014). Birth Prevalence of Congenital Cytomegalovirus Among Infants of HIV-Infected Women on Prenatal Antiretroviral Prophylaxis in South Africa. Clinical Infectious Diseases.m58:1467–72.

Olusanya BO, Slusher TM, Boppana SB (2015). Prevalence of congenital cytomegalovirus infection in Nigeria: a pilot study. Pediatric and Infectious Diseases Journal. 34(3):322-4. doi: 10.1097/INF.0000000000000555. PMID: 25742080; PMCID: PMC4352715.

Mayaphi SH, Brauer M, Morobadi DM, Mazanderani AH, Mafuyeka RT, Olorunju SAS (2014). Cytomegalovirus Viral Load Kinetics in Patients with HIV/AIDS Admitted to a Medical Intensive Care Unit: A Case for Pre-Emptive Therapy. PLoS One. 9: 93702.

Van der Sande MA, Kaye S, Miles DJ, Waight P, Jeffries DJ, Ojuola OO, Palmero M, Pinder M, Ismaili J, Flanagan KL, Aveika AA, Zaman A, Rowland-Jones S, McConkey SJ, Whittle HC, Marchant A (2007). Risk factors for and clinical outcome of congenital cytomegalovirus infection in a peri-urban West-African birth cohort. PLoS One. 6;2(6):492. doi: 10.1371/journal.pone.0000492. PMID: 17551573; PMCID: PMC1876257.

Hsiao NY, Zampoli M, Morrow B, Zar HJ, Hardie D (2013). Cytomegalovirus viraemia in HIV exposed and infected infants: prevalence and clinical utility for diagnosing CMV

pneumonia. Journal of Clinical Virology.58(1):74-8. doi: 10.1016/j.jcv.2013.05.002. PMID: 23727304.

Karimian, P., Yaghini, O., Nasr Azadani, H., Mohammadizadeh, M., Arabzadeh, S. A., Adibi, A., & Rahimi, H. (2016). Prevalence, Characteristics, and One-Year Follow-Up of Congenital Cytomegalovirus Infection in Isfahan City, Iran. Interdisciplinary perspectives on infectious diseases, 7812106. https://doi.org/10.1155/2016/7812106Q

Zhang XW, Li F, Yu XW, Shi XW, Shi J, Zhang JP (2007). Physical and intellectual development in children with asymptomatic congenital cytomegalovirus infection: a longitudinal cohort study in Qinba mountain area, China. Journal of Clinical Virology. 40:180–5.

Forner G, Abate D, Mengoli C, Palù G, Gussetti N (2015). High Cytomegalovirus (CMV) DNAemia Predicts CMV Sequelae in Asymptomatic Congenitally Infected Newborns Born to Women with Primary Infection During Pregnancy. Journal of Infectious Diseases. 212: 67-71

ENTOMOCIDAL EFFECT OF SOME ESSENTIAL OILS: AN ALTERNATIVE TO SYNTHETIC PESTICIDES IN THE CONTROL OF *DERMESTES MACULATUS* DEGEER 1774 (COLEOPTERA: DERMESTIDAE)

Nasiru*, A.
Suleiman, M.
Department of Biology, Umaru Musa Yar'adua University Katsina, Nigeria

ABSTRACT
Realizing the hazardous effect of synthetic pesticides to human health, non-target organism and environment aroused the attention of scientific community in providing alternative pesticides which are natural, safe and effective. In this study, *Thymus vulgaris* L and *Syzygium aromaticum* L essential oils (TEO and EOSA) were evaluated against *Dermestes maculatus* under laboratory conditions by residual contact method. The oils were obtained by steam distillation using a modified Clevenger apparatus. Four concentrations (2.5, 5, 7.5 and 10%) of each of the essential oils (EOs) were prepared using acetone as a solvent and separately applied on 15 g of smoke-dried *C. gariepinus*. Newly emerged adults, 3rd instar larvae and newly laid eggs of the pest were exposed to the treated fish samples for a period of five days. Results showed that both oils had varying degrees of larvicidal and adulticidal properties against *D. maculatus*. Similarly, the EOs significantly inhibited oviposition, egg hatchability and adult emergence of the pest. In all cases EOSA was the most effective which resulted in highest effects at all concentrations with lowest LC_{50} and LT_{50}. Therefore EOSA and TEO could be used in the control of *D. maculatus* infesting smoke-dried *C. gariepinus.*
Keywords: *Clarias gariepinus*, *Dermestes maculatus*, Essential oils, Synthetic pesticides, *Syzygium aromaticum*, *Thymus vulgaris*, Infestation

INTRODUCTION
Clarias gariepinus is the most common fish species preserved by smoke-drying in most part of Nigeria (Ayeloja *et al.,* 2015). It is highly nutritious, containing high amount of vitamins, proteins, minerals, no saturated fat, and is low in carbohydrates. Unique taste, flavor and texture of *C. gariepinus* makes it widely accepted in most part of Nigeria (Aremu *et al*., 2013). Smoking is the most popular method of fish processing among fish mongers in Nigeria and perhaps the simplest method as it does not require sophisticated equipment or highly skilled labor (. Smoke-dried fish is a good alternative to fresh fish and their nutritional values are not significantly different (Ajao *et al*., 2018). Despite the preservation method adopted, *Dermestes maculatus* (Deeger 1774) L, accounts for about 71.5% of smoke-dried fish infestation in most of the producing areas in Nigeria (Babarinde *et al*., 2018; Ayeloja *et al*, 2020; Indabo and Zakari, 2020). The infestation which causes 50% - 62.5% qualitative and quantitative loss as well as declining in consumer acceptability and market prices of smoke-dried *C. gariepinus* (Babarinde *et al*., 2018)
Management of insect pests in many storage systems relies primarily on applying synthetic insecticides due to their effectiveness, relative long shelf life and application. However, misuse and over use of these chemicals resulted in their harmful effects on human health, non-target members of the ecosystem, environment, resistance development and persistent residues. Based on FAO/WHO MRLs recommendations, food stuff acceptable for human consumption must be free from insect exuvia, off smell, unusual colour, mycotoxins and must not contain active substances of pesticides above the maximum residue limits. Because consuming pesticides in any form could lead to acute or chronic discomfort. However,

scientific reports shows residues of pesticides such as DDT, Captan, Hexazinone, and Tecnazene were all found above the recommended limit in a smoke-dried *C. gariepinus* (Adedolapo, 2018; Dauda *et al*., 2019; Manuel *et al*., 2020; Oyinloye *et al.,* 2021). Therefore realization of the serious limitations offered by the use of these chemicals, aroused the attention of scientific community on seeking alternative methods of controlling insect pests which is safe for humans, animals and the environment (Garay, 2020). One of such promising development is in the use of plant-derived materials such as powders, extracts and essential oils (Akinwumi, 2017; Trivedi et al., 2017; Babarinde *et al*., 2018; Nta *et al*., 2019; Soe *et al.*, 2020; Indabo and Zakari, 2020; Georgina *et al*., 2020; Moustapha *et al*., 2021; Nasiru *et al*., 2022; Suleiman and Nasiru 2022).

Essential oils (EOs) are volatile oils, found mostly in aromatic plants as secondary metabolites consisting a mixtures of natural organic compounds which play an important role in plants defense system against microorganisms, insects and herbivores. EOs are soluble in organic solvents and can be extracted from seeds, buds, stems, leaves and flowers (Ravi *et al*., 2019). They show wide and varied bio - activities against both agricultural and medically important insect pests, ranging from toxicity with ovicidal, larvicidal, pupicidal and adulticidal activities to sub-lethal effects including oviposition deterrence, antifeedant and repellence actions (Campolo 2018). They were reported to have the capacity of interfering with basic metabolic, biochemical, physiological and behavioral functions of insects. Meanwhile, they are selective and have little or no harmful effect on human health, non-target and environment (Ebadollahi and Mahboubi, 2011).

The essential oils of *S. aromaticum* and *Cinnamomum zeylanicum* resulted in insecticidal activities similar to Deltamethrin against *Callosobruchus maculatus* in a dosage dependent manner. Application of the oils, proportionately delayed growth rate of the pest, inhibit oviposition among parents exposed to LD_{20} of each essential oils and significantly impaired offspring emergence (Jumbo *et al.,* 2018). Similarly, essential oil obtained from leaves of clove presented effective larvicidal and antifeedant activities against 3rd instar larvae of the armyworm (*Spodoptera litura)* at 2.0% concentration with LC_{50} of 0.09% and LT_{50} of 24.6h (Fateha *et al*., 2021). Moreover, Clove essential oil was reported to cause 100% mortality of *Sitophilus zeamais* and *A. obtectus* at a concentrations of 17.9 and 35µL g-1, 48 h after treatment (Jairoce *et al.,* 2016). In addition, when tested against *Callosobruchus chinensis* using fumigation method. The essential oil of *S. aromaticum* recorded lower LC_{50} values (0.334, 0.341 and 0.059ml/cm^2) after 24hrs, 48hrs and 72hrs, respectively (Trivedi *et al.,* 2017).

Furthermore, application of 1% *Thymus vulgaris* essential oil, commercial thymol and carvacrol yeilded 50.00%, 86.67% and 85.00% larval mortality of lesser mealworm (*Alphitobius diaperinus)* under laboratory conditions. The mortality increased to 62.5%, 91.67% and 97.5%, with increasing concentrations of the treatments to 2% (SZCZEPANIK *et al*., 2012). Also, the essential oil of *Thymus vulgaris* was reported to have least LC_{50} among five essential oils tested, using leaf dipping and spray bioassay methods against *Pochazia shantungensis* adults (57.48 and 75.80 mg/L) and nymph (56.74 and 28.52 mg/L), respectively (Park *et al*., 2017). Moreover, the essential oil of *Thymus vulgaris* resulted in reduction of adult survival, deterred oviposition and longevity of *Acanthoscelides obtectus* in a concentration-dependent manner using residual contact toxicity (Lazarevi´c *et al*., 2020). Meanwhile, significant (56%) adult mortality of *Callosobruchus maculatus* was recorded in 0.25 µl of thyme essential. Further increase in concentration to 30 µl resulted in 100% of the pest (Estekhdami *et al*., 2020). Although, essential oils have been found to be an important source of bio-pesticides, majority of the studies were mostly on another insects other than *D. maculatus.* . Therefore, the aim of this research was to assess entomocidal effect of some essential oils against *Dermestes maculatus* infesting smoke-dried *Clarias gariepinus.*

MATERIALS AND METHODS

Collection and Preparation of Plants Materials

The spices used for this study (Plate I) were purchased from Fatima Baika Central Market Katsina, formally identified and voucher numbers were assigned to each, by plant taxonomist at the Department of Biology, Umaru Musa Yar'adua, Katsina (UMYUK). The spices were then washed using tap water and air dried under shade for a period of five days in the laboratory and kept separately in a well labeled plastic containers (Soe *et al*., 2020).

Plate I: Spices used for the study (A) *T. vulgaris* leaves; (B) *S. aromaticum* buds

Extraction of the essential oils

Essential oil of each spice was extracted by steam distillation, at Chemistry Laboratory of UMYUK, using Clevenger apparatus with some modifications. Seventy five grams (75 g) leaves and buds of *T. vulgaris* and *S. aromaticum* were separately taken into a 1000 mL round bottom flasks, and 750 mL of distilled water was added. The flasks were heated on heating mantle for a period of three hours. The extracted essential oils was evaporated along with water vapor, through a connection tube to condenser. Distillate was collected in a separation funnel and water was drained into a beaker leaving the top layer of the essential oil in the tube. Excess water was removed by passing the oils over anhydrous sodium sulfate and then kept in a dark sealed vials at 4 ^{0}C (Ileke *et al*., 2020)

Collection and Preparation of Fish sample

Un-infested, seasonings and treatment free smoke-dried *C. gariepinus* (Plate II) with no visible presence of neither adult or larvae of *D. maculatus* was purchased from fish vendors at Fatima Baika Central Market Katsina. The fish sample was transported to Postgraduate Laboratory of the Department of Biology, UMYUK were it was weight and disinfested in dry air oven at 60^{0}C for 60 minutes to kill all possible insect pests and their eggs that might be present and later allow to cool at room temperature in the laboratory (Adesina *et al*., 2015).

Rearing of *Dermestes maculatus*

The initial source of *D. maculatus* used for this study were obtained from naturally infested smoke-dried *C. gariepinus* and in wall cracks of dry fish vendor's shops at Fatima Baika Central Market Katsina, Katsina State of Nigeria. The beetles were taken to Postgraduate Laboratory of the Department of Biology UMYUK, in a plastic jars covered with muslin cloth (Ileke, 2021). Twenty pairs of identified and confirmed Adult *D. maculatus* were introduced into three different rearing containers containing water soaked cotton wool, 50 g disinfested smoke-dried *C. gariepinus* free of seasoning, salt and or treatments. To meet water requirement for oviposition and source of food to the beetles (Bello and Lawan, 2021). The

containers were then covered with muslin cloth, to prevent escape of the insects and or entrance of unwanted one. The set up was then kept in an incubator at a temperature of 28 ± 2^0C and 65 ± 5% relative humidity (R.H) for a period of three weeks to allow oviposition and larval emergence. New generation was prepared by removing newly emerged larvae from the stock culture and placed on another prepared set up (Ileke, 2021)

Larvicidal Bioassay

To test larvicidal activity of *S. aromaticum, T. vulgaris* and *C. citratus* essential oils against *D. maculatus*. Residual contact method adopted by Soe *et al.* (2020) was employed. Four stock solutions (2.5, 5.0, 7.5 and 10% concentrations) of each selected essential oils. Were thoroughly sprayed using 5 ml syringe, on 15 grams of disinfested smoke-dried *C. gariepinus* placed in a labeled transparent plastic containers. Same grams of *C. gariepinus* treated with acetone only served as a control. The treated and control samples were air-dried for 2 hours to allow solvent evaporation. Ten 3rd instar larva of *D. maculatus* were released on each treated fish flesh in the containers and covered with perforated lid fitted with muslin cloth to allow aeration and to prevent entrance of some insects from outside. All treatments and control were replicated three times and kept in an incubator at 28^0C and 65 ± 5% R.H. Larval mortality was recorded every 24 for 4 days whereby larva was judged dead when failed to respond to abdominal Pin-punch and mean percentage larval mortality was calculated using the following formula:

$$\text{Larval Mortality (\%)} = \frac{\text{Number of Dead Larvae}}{\text{Number of Larvae Introduced}} \times 100$$

Adulticidal Bioassay

To test the toxicity of *S. aromaticum, T. vulgaris* and *C. citratus* essential oils against adult *D. maculatus*. Residual contact method employed by Soe *et al*. (2020) was adopted. Fifteen grams (15 g) of disinfested, seasoning and treatment free smoke-dried *C. gariepinus*, placed in labeled transparent plastic containers were thoroughly sprayed, with 5 ml of each concentration of the essential oils. A control was also prepared by spraying 5ml of acetone only on the same grams of smoke-dried fish. Each of the treatments and control were replicate three times. The treated samples and control, were air-dried for 2 hours to allow the solvent evaporation. Five pairs of newly emerged (0-24 hours old) adults *D. maculatus* were then released into each container containing and a water soaked cotton wool to meet water requirement and covered with perforated lids fitted with net from inside. To allow aeration, prevent insects from escape and to stop intrusion of other insects from outside. The containers were then kept at laboratory conditions for five days. Adult mortality was recorded every 24 hours, insects were judged dead when they failed to respond to pin probe and percentage mortality was calculated using below formula:

$$\text{Adult Mortality (\%)} = \frac{\text{Number of dead Adults}}{\text{Number of Adults introduced}} \times 100$$

Examination of oviposition, Egg hatchability and Adults emergence of *D. maculatus* on smoke-dried *C. gariepinus* treated with the selected essential oils

To examine effect of *S. aromaticum, T. vulgaris and C. citratus* essential oils on Oviposition, egg hatchability and adult emergence of *D. maculatus*. Fifteen grams (15 g) of smoke-dried *C. gariepinus* placed in a labeled plastic containers, were thoroughly sprayed with 5 ml of the essential oils at 2.5%, 5.0%, 7.5% and 10% concentrations. The treated fish samples were air dried for 2 hours to allow evaporation of the solvent. Ten pairs of newly emerged (0 -24 hours) *D. maculatus* adults were introduced into each container and a water soaked cotton wool was placed to meet the water requirement for oviposition. The containers were then covered with a perforated lids fitted with net. To allow aeration, prevent escaping of the

beetles and or intrusion of other insects from outside. Same weight of *C. gariepinus* treated with acetone only served as control and all containers were replicated three times. The number of eggs laid were counted after 24 hours, 48 hours and 72 hours using hand lens and soft entomological brush, after which, all dead and alive adult beetles were removed from the treated and control containers Percentage reduction of egg laid was calculated using the following formula (Adesina *et al*., 2015)

$$\text{Oviposition Deterrence (\%)} = \frac{\text{NEC} - \text{NET}}{\text{NEC}} \times 100$$

Where: NEC = Number of eggs laid in the control and NET = Number of eggs laid in the treated samples.

Further, fourty (40) newly laid eggs were introduced into another set of containers containing same grams of *C. gariepinus* and treatment. Treated and control containers were replicated three times and kept in an open air shelf at laboratory conditions. The number of eggs hatched were recorded at 48, 72 and 96 hours after treatment. Percentage hatchability was determined using the following formula (Adesina *et al*., 2015)

$$\text{Egg Hatchability (\%)} = \frac{\text{Number of Eggs Hatch}}{\text{Number of Eggs Introduced}} \times 100$$

In a similar experimental set up, thirty 3rd instar larvae were introduced into each container containing 15 g of *C. gariepinus* treated with concentrations of the EOs. A transparent plastic container with same weight of fish sample treated with acetone only served as a control. Both treated and control were replicated three times and kept at a laboratory conditions. The number of larva reaching adult stage were recorded in both treated and control containers after four weeks and the percentage reduction in adult emergence of F_1 progeny or inhibition rate (IR) was calculated by the following formula (Suleiman *et al*., 2019).

$$\text{Inhibition Rate (\%)} = \frac{\text{Cn} - \text{Tn}}{\text{Cn}} \times 100$$

Where: Cn is the number of emerged insects in the control and Tn is the number of emerged insects in the treated containers.

Data Analysis

Mortality data were subjected to one way Analysis of Variance (ANOVA) at $P<0.05$ to determine any significant difference among the essential oils. Tukey HSD was employed to separate the means. Mortality data from all the oils were subjected to Probit Analysis using SPPS 23 version to determine their median lethal concentration (LC_{50}) and lethal time (LT_{50}) of the essential oils.

RESULTS

Larvicidal effect of the tested essential oils against *Dermestes maculatus*

Mean percentage larval mortality of *D. maculatus* exposed to *C. gariepinus* treated with varying concentrations of *Syzygium aromaticum* essential oil (EOSA) is presented in Figure 4.1. Result shows that 24 hours after exposure (HAE), highest (80.00%) larval mortality was recorded in both 7.5 and 10.0% concentrations while lower (23.30 and 40.00%) mortality was observed in 2.5 and 5.0% concentrations, respectively. Furthermore, 48 HAE the mortality increased to 70.00% in 5.0% and 90.00 - 100% in *C. gariepinus* treated with 7.5 - 10% concentrations of EOSA. Meanwhile, with extending the exposure period to 72 – 96 hours, larval mortality increased on smoke-dried *C. gariepinus* treated with 2.5% (50.00%) and 5.0% (83.30%) concentrations of EOSA, respectively (Figure 1).

Result of larval mortality of *D. maculatus* on smoke-dried *C.gariepinus* treated with varying concentrations of *Thymus vulgaris* essential oil (TEO) is presented in Figure 4.2. The result highlighted that, lower (10% and 20%) larval mortality of *D. maculatus* was recorded on *C. gariepinus* treated with 7.5% and 10% concentrations of TEO 24 HAE. Meanwhile, the

mortality increased to 40% and 50% in these concentrations 48 HAE. The figure further shows that, with the extension of the exposure hours to 72 – 96 hours, 6.67% - 30% and 70% - 93.33% mortalities were recorded in 2.5% - 5.0% and 7.5 - 10% concentrations of TEO, respectively (Figure 2).

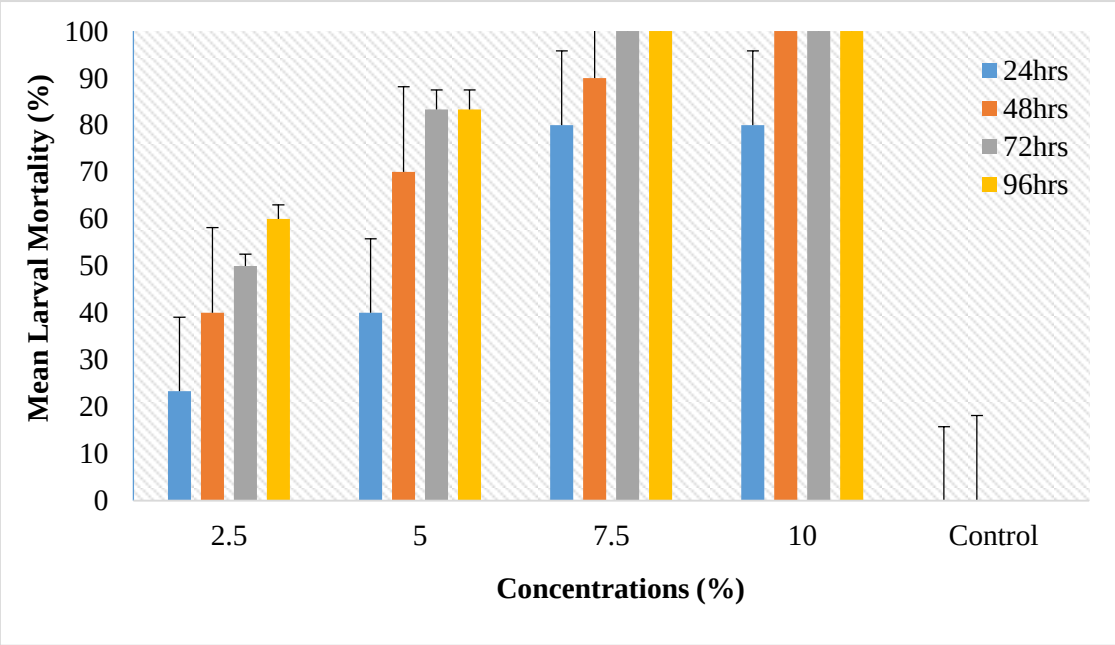


Figure 1: Larval Mortality of *D. maculatus* in varying concentrations of EOSA

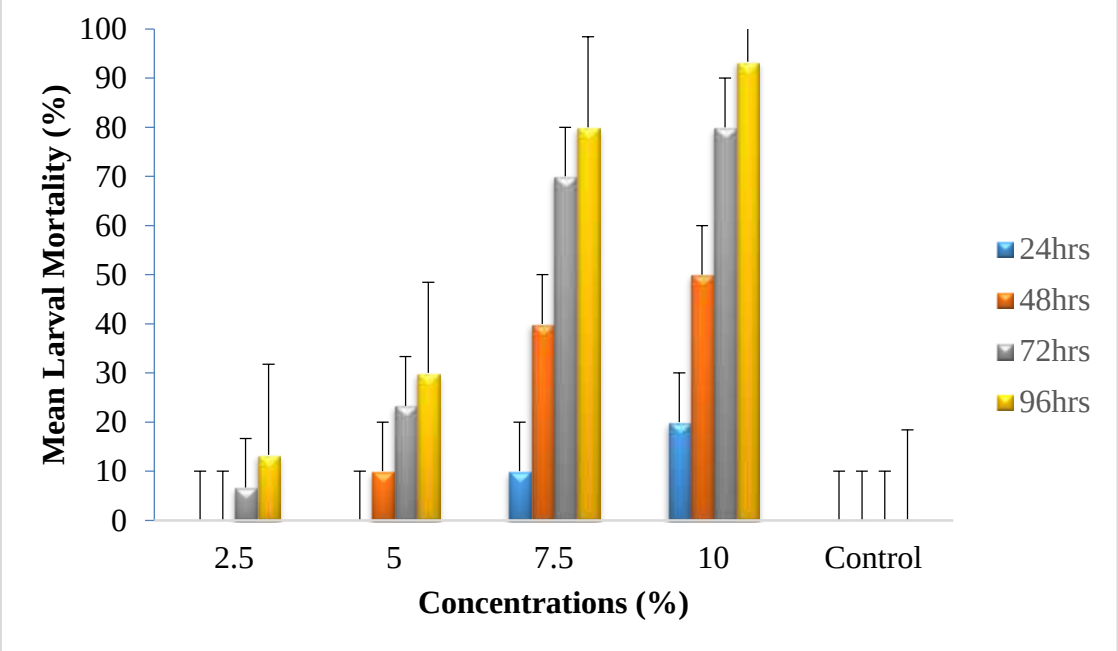


Figure 2: Larval Mortality of *D. maculatus* in varying concentrations of TEO

Adulticidal effect of the tested essential oils against *Dermestes maculatus*

Mortality result of adults *D. maculatus* in contact with *C. gariepinus* treated with varying concentrations of EOSA is presented in Figure 3. The result shows that, 50.00, 66.67, 86.67 and 100% mortality of the adult *D. maculatus* was recorded on smoke-dried *C. gariepinus* treated with 2.5, 5.0, 7.5 and 10% of EOSA, respectively 24 HAE. Furthermore, with extension of exposure period to 48 - 72 hours, the mortality increased to 70.00 - 100% on *C.*

gariepinus treated with 2.5 - 7.5% concentrations of EOSA. , complete (100%) motality was recorded in all concentrations of EOSA, 96 HAE (Figure 3).
Similarly, mortality result of adult *D. maculatus* exposed to smoke-dried *C. gariepinus* treated with varying concentrations of TEO is presented in Figure 4.4. Result revealed that 80.00% and 90.00% of the exposed adults died in 7.5 and 10% concentrations while, only 10.00% and 30.00% observed dead in 2.5 and 5% concentrations, respectively, 24 HAE (Figure 4.4). However, the mortality increased to 50.00% in 5.0% concentration and 100% in fish samples treated with 7.5% and 10% concentrations, respectively 48 HAE. Moreover, at 72 and 96 HAE, the mortality increased to 70.00% and 80.33%, respectively, in *C. gariepinus* treated with 2.5 and 5.0% of TEO, respectively (Figure 4).

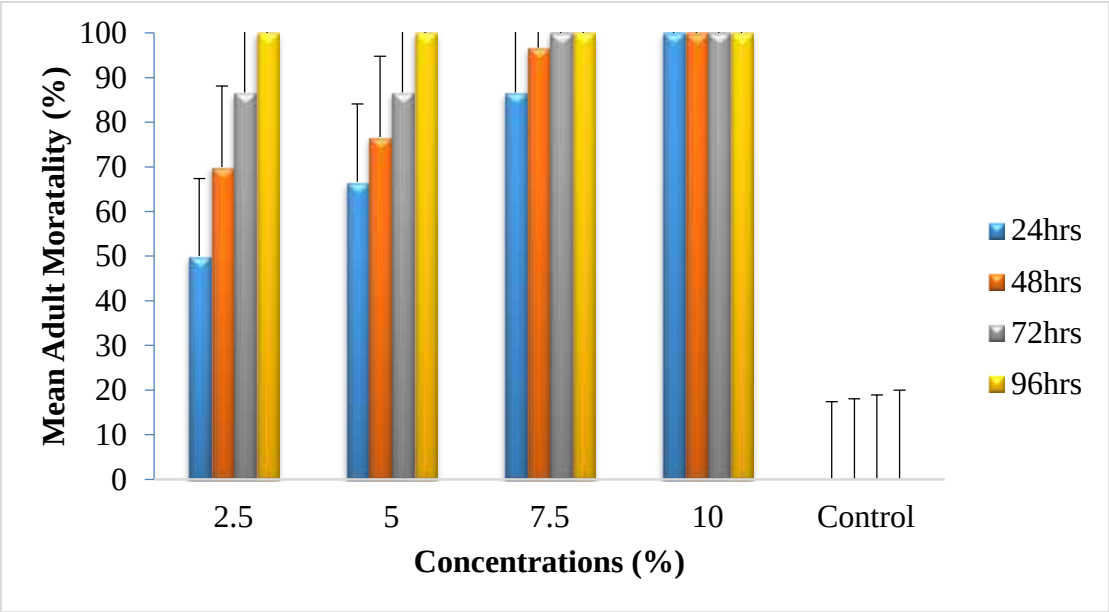


Figure 3: Adult mortality of *D. maculatus* varying concentrations of EOSA

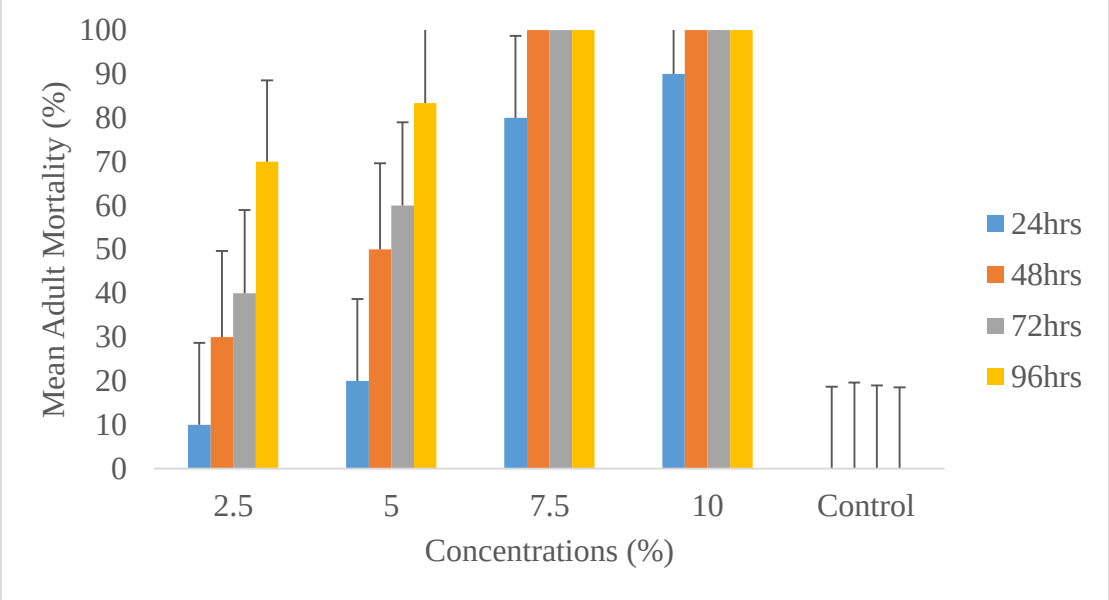


Figure 4: Adult Mortality of *D. maculatus* in varying Concentrations of TEO

Median lethal concentration (LC_{50}) and lethal time (LT_{50}) of EOSA, TEO against *D. maculatus*

The concentration of essential oil needed to kill 50% of adult *D. maculatus* (LC_{50}) varied significantly among the EOs tested. EOSA had the lowest LC_{50} of 2.72, 1.80 and 0.99%, while TEO exhibited higher LC_{50} of 5.71, 3.74 and 3.43% concentrations at 24, 48 and 72 hours, respectively (Table 1). Similarly, the LC_{50} of the EOs against larva of *D. maculatus* is lower (4.87, 3.14 and 2.60%) in EOSA and higher (13.93, 9.66 and 6.30% in TEO, respectively at the same period of time (Table 2).

In addition, EOSA took 24.04, 17.15, 10.94 and 7.00 hours to kill 50% of the adult *D. maculatus* (LT_{50}) at 2.5, 5.0, 7.5 and 10% concentrations respectively, while TEO required 73.43, 49.55, 13.55 and 9.63 hours to cause the same effect at the same concentrations (Table 1). On the other, EOSA took 69.04, 29.70, 13.10 and 7.00 hours to kill 50% of *D. maculatus* larvae at the exposed concentrations, while TEO required 158.01, 131.24, 54.56, 13.55 and 43.87 hours to cause the same effect at the same concentrations (Table 2).

Table 1: Median Lethal Concentration (LC_{50}) and Lethal Time (LT_{50}) of EOSA and TEO against Adult *D. maculatus*

Essential Oils	LC_{50} (%)			LT_{50} (Hours)			
	Exposure Period (Hours)			Concentrations (%)			
	24	48	72	2.5	5	7.5	10
EOSA	2.72	1.80	0.99	26.40	17.15	10.94	7.00
TEO	5.71	3.74	3.34	73.43	49.55	13.55	9.63

Table 2: Median Lethal Concentration (LC_{50}) and Lethal Time (LT_{50}) of EOSA and TEO against *D. maculatus* Larva

Essential Oils	LC_{50} (%)			LT_{50} (Hours)			
	Exposure Period (Hours)			Concentrations (%)			
	24	48	72	2.5	5	7.5	10
EOSA	4.87	3.14	2.60	69.04	29.70	13.10	13.50
TEO	13.93	9.66	6.30	158.01	131.24	54.56	43.87

KEY: EOSA= Essential oil of *S. aromaticum*, TEO= *T. vulgaris* Essential oil

Effect of the tested essential oils on oviposition, eggs hatchability and Adults emergence of *D. maculatus*

Table 3, shows that there is no significant differences ($P > 0.05$) in effects of the varying concentrations of the tested essential oils on oviposition, eggs hatchability and adult emergence of *D. maculatus*. Further, the result revealed higher oviposition deterrence in 2.5% and 5.0% concentrations of EOSA (89.96% and 92.41%) and TEO (82.40% and 92.06%). Also, total (100%) deterrence was recorded on smoke-dried *C. gariepinus* treated with 7.5 and 10% concentrations of both EOSA and TEO. Moreover, the table presented that few eggs were able to hatch in both 2.5% and 5.0% concentrations of the essential oils. While egg hatchability was completely (100%) halted in 7.5% and 10% concentrations of these EOs (Table 3). Meanwhile, majority (90.34% - 97.41%) of the *D.maculatus* larva exposed to smoked-dried *C.gariepinus* treated with 2.5% - 5.0% concentrations of EOSA and TEO, were inhibited to emerge as adult. Similarly, 98.24% - 100% adult emergence inhibition was observed on *C.gariepinus* treated with 7.5% - 10% concentrations of EOSA and TEO (Table 3)

Table 3: Effect of the tested essential oils on oviposition, eggs hatchability and adult emergence of *D. maculatus*

Concentrations (%)	Essential oils	Number of eggs laid	Oviposition deterrence (%)	Number of eggs Hatched	Egg Hatchability (%)	Number of Adults emerged	Emergence Inhibition rate (%)
2.5	EOSA	9.67±0.33^{a}	89.96	2.67±0.33^{a}	6.68	1.00±0.00^{a}	97.41
	TEO	17.00±0.58^{a}	82.40	3.33±0.33^{a}	8.33	3.67±0.33^{a}	90.34
5.0	EOSA	7.33±0.33^{a}	92.41	2.00±0.00^{a}	5.00	1.00±0.00^{a}	97.41
	TEO	7.67±0.33^{a}	92.06	3.00±0.00^{a}	7.50	2.67±0.33^{a}	92.97
7.5	EOSA	0.00±0.00^{a}	100.00	0.00±0.00^{a}	0.00	0.00±0.00^{a}	100.00
	TEO	0.00±0.00^{a}	100.00	0.00±0.00^{a}	0.00	0.67±0.33^{a}	98.24
10.0	EOSA	0.00±0.00^{a}	100.00	0.00±0.00^{a}	0.00	0.00±0.00^{a}	100.00
	TEO	0.00±0.00^{a}	100.00	0.00±0.00^{a}	0.00	0.00±0.00^{a}	100.00
Control		96.56±0.64^{c}	0.00	39.56±0.14^{c}	98.9	38.00±0.00^{c}	5.00

Means followed by the same letter(s) in the same column are not significantly different (P > 0.05) from each other using Turkey's HSD Test

DISCUSSION

The findings of this study revealed the entomocidal effect of the essential oils of *S. aromaticum* and *T. vulgaris* against *D. maculatus* infesting smoke-dried *C. gariepinus.* Both tested essential oils shows significant larvicidal, adulticidal, ovicidal effects. The oils also impaired oviposition and inhibited adult emergence of *D. maculatus*. Both essential oils were effective at all concentrations with lowest LC_{50} and LT_{50} against adult and larva of the pest. The adulticidal and larvicidal effect of the selected essential oils confirmed the findings of Wilane *et al*. (2014) who reported 70% and 90% mortality of 1st instar larvae of *D. maculatus, and D. frischii* exposed to 0064µl/ml and 0.512µl/ml concentrations of *Mentha spicata* using fumigation method 24 hours of exposure. The findings also support the report of Estekhdami *et al*. (2020) that 0.25 µl of thyme essential resulted in significant (56%) mortality of adult *Callosobruchus maculatus* and further increase in concentration of the oil to 30 µl resulted in 100% of the pest.

In addition, result of the present study revealed the effectiveness of the tested essential oils in oviposition deterrence, inhibition of egg hatchability and adult emergence. This is in-line with the findings of Jumbo *et al.* (2018) who reported proportional delay in growth rate, oviposition deterrence and significant impairment of offspring emergence of *Callosobruchus maculatus* exposed to LD_{20} of the essential oils of *S. aromaticum* and *Cinnamomum zeylanicum.* Similarly, the findings is in agreement with the report of Lazarevi´c *et al*. (2020) that, the essential oil of *Thymus vulgaris* resulted in reduction of adult survival, deterred oviposition and longevity of *Acanthoscelides obtectus* in a concentration-dependent manner using residual contact toxicity.

Conclusion

Base on the findings of this study it could be inferred that both essential oils have effective insecticidal properties against adult, larva, oviposition, egg hatchability and adult emergence of *D. maculatus.* Lower LC_{50} of EOSA revealed it is high efficiency against the pest. Therefore the tested essential oils could play a vital role in management and control of *D. maculatus* infesting smoke-dried *C. gariepinus*.

REFERENCES

Adedolapo, . O. (2018). Quantification of Organophosphate and Carbamate residue on stored grains in Ondo State, Nigeria. *Journal of Biology and Medicine*, *2*(1), 001-006. 00

Ajao, A. M., Oladipo, S. O., and Soliu, B. O. (2018). *Dermestes maculatus* Degeer infestation impact on market loss of dried fish in Kwara State, Nigeria. *Aquaculture and Fisheries*, *3*(4), 170-173

Ajayi, O. E., Oladele, O. O., and Fabunmi, G. O. (2019). Differential inhibitory potentials of three plant extracts against *Dermestes maculatus* Degeer and microorganisms in smoked catfish, *Clarias gariepinus* Burchell. *Food Quality and Safety*, 3(3), 201-208.

Akinwumi, F. O., and Fesobi, M. E. (2016). Effect of some plant-based insecticides on the nutritive value of smoked catfish, *Clarias gariepinus* (Pisces: Clariidae). African Journal of Food Science, 4(4), 143-147.

Ayeloja, A. A., George, F. O. A., Akinyemi, A. A., and Atanda, O. O. (2015). Proximate and mineral composition of spiced, smoked catfish Clarias gariepinus (Burchell, 1822). *Journal of Agricultural Science and Environment*, *15*(2), 68-74.

Babarinde, S. A., Sunnie-Ododo, M. O., Akanbi, W. B., Oyegoke, O. O., Tijani, R., and Olaobaju, S. F. (2018). Comparative susceptibility of two developmental stages of hide beetle (*Dermestes maculatus* Degeer, 1774) to ginger (*Zingiber officinale* Roscoe) essential oil. *Journal of the Saudi Society of Agricultural Sciences*, *17*(4), 385-389.

Bello, M. M., and Lawan, E. A. (2021). The effect of different concentrations of water extract of Sodom apple leaves (*Calotropis procera*) I in the control of *Dermestes maculatus* on smoked fish (*Oreochromis niloticus* Linnaeus, 1758).

Bob-Manuel, R. B., Egbecho, E., and Zakka, U. (2020). Death in the Mouth: The Current Status of Pesticide Residues in Cured Fish from Selected Markets in Rivers State Nigeria. International Research Journal of Insect Sciences, 5(1), 14-22.

Campolo, O., Giunti, G., Russo, A., Palmeri, V., and Zappalà, L. (2018). Essential oils in stored product insect pest control. *Journal of Food Quality*.

Dauda, A. M., Nasiru A., and Suleiman. M (2019). The impact of some agricultural practices on population status of honey bee, *Apis mellifera* L in Katsina State, Nigeria. *International journal of entomology research*, *40*(10), 9-90.

Ebadollahi, A., and Jalali Sendi, J. (2015). A review on recent research results on bio-effects of plant essential oils against major Coleopteran insect pests. Toxin Reviews, 34(2), 76-91

Estekhdami, P., Dehsorkhi, A. N., and Kalvandi, R. (2020). Insecticidal efficacy of essential oils from *Cinnamomum zeylanicum*, *Thymus vulgaris*, *Ferula assafoetida* L on *Callosobruchus maculatus* F. *Asian Journal of Advances in Agricultural Research*, 52-62.

Fateha, R. N., Grasela, M., Ichwan, M. N., Purwanti, E. W., and Kurniasari, I. (2021). Larvicidal and antifeedant activities of Clove leaf oil against *Spodoptera litura* (F.) on soybean. *jurnal hama dan penyakit tumbuhan tropika*, 21(1), 20-25.

Garay, J., Brennan, T., and Bon, D. (2020). Essential Oils A Viable Pest Control Alternative. International Journal of Ecotoxicology and Ecobiology, 5(2), 13

Ileke, K. D. (2021). Responses of two plant-derived bio-insecticides as protectants of smoke-dried catfish, *Clarias gariepinus* [Pisces: Clariidae] against hide beetle, *Dermestes maculatus* (De Geer) [Coleoptera: Dermestidae]. Bulletin of the National Research Centre, 45(1), 1-9.

Ileke, K. D., Adesina, J. M., Abidemi-Iromini, A. O., and Abdulsalam, M. S. (2021). Entomocide effect of *Alstonia boonei* De wild on reproductive performance of *Dermestes maculatus* (Coleoptera: Dermestidae) infestation on smoked catfish *Claria gariepinus* (Pisces: Clariidea). *International Journal of Tropical Insect Science*, 41(2), 1293-1304

Indabo, S. S., and Zakari, R. (2020). Phytochemical screening and larvicidal effect evaluation of *Azadirachta indica* (L) leaf extract on *Dermestes maculatus* (de geer, 1774) infestation of smoked *Clarias gariepinus* (burchell, 1822). *Fudma journal of sciences*, 4(2), 41-45.

J G, M RB, S SMD (2020). Developmental impairment and Oviposition deterrence in *Callosobruchus maculatus* (Fabricius) infesting *Vigna radiate* (L.Wilczeck) upon treatment with five different plant products. *Indian Journal of Science and Technology* 13(16): 1648-1655. https://doi.org/10.17485/IJST/v13i16.470

Jairoce, C. F., Teixeira, C. M., Nunes, C. F., Nunes, A. M., Pereira, C. M., and Garcia, F. R. (2016). Insecticide activity of clove essential oil on bean weevil and maize weevil. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 20(1), 72-77.

Lazarević, J., Jevremović, S., Kostić, I., Kostić, M., Vuleta, A., Manitašević Jovanović, S., and Šešlija Jovanović, D. (2020). Toxic, oviposition deterrent and oxidative stress effects of *Thymus vulgaris* essential oil against *Acanthoscelides obtectus*. Insects, 11(9), 563.

Moustafa, M. A., Awad, M., Amer, A., Hassan, N. N., Ibrahim, E. D. S., Ali, H. M., and Salem, M. Z. (2021). Insecticidal activity of lemongrass essential oil as an eco-friendly agent against the black Cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae). *Insects*, 12(8), 737

Nasiru, A., Suleiman, M., and Abdulkarim, B. (2022). Bio-efficacy of the essential oil of *Thymus vulgaris* against *Dermestes maculatus* Degeer 1774 (Coleoptera: Dermestidae) infesting smoke-dried *Clarias gariepinus* Burchell 1822. *The Bioscientist Journal*, *10*(3), 270-279.

Nta, A. I., Andem, A. B., Okererke, C. I., and Odey, C. O. (2019). Bio-efficacy of some plant oil extracts as surface protectant against leather beetle, *Dermestes maculatus* (Degeer) on smoked fish of *Clarias gariepinus*

Oyinloye, J. A., Oyekunle, J. A. O., Ogunfowokan, A. O., Msagati, T., Adekunle, A. S., and Nety, S. S. (2021). Human health risk assessments of organochlorine pesticides in some food crops from Esa-Oke farm settlement, Osun State, Nigeria. *Heliyon*, *7*(7), e07470.

Park, J. H., Jeon, Y. J., Lee, C. H., Chung, N., and Lee, H. S. (2017). Insecticidal toxicities of carvacrol and thymol derived from *Thymus vulgaris* Lin. against *Pochazia shantungensis* Chou and Lu, newly recorded pest. *Scientific reports*, *7*(1), 1-7.

Ravi, Y., Meena, N. K. and Lal, G. (2019). Spices: A novel source for insect-pest management.

Soe, T. N., Ngampongsai, A., and Sittichaya, W. (2020). Bioactivity of some plant essential oils for seed treatment against pulse beetle, *Callosobruchus maculatus* F (Coleoptera: Bruchidae) on mung bean. Bulgarian Journal of Agricultural Science, 26(1), 141-147.

Suleiman, M., and Nasiru, A. (2022). Bio-activity of the essential oil of *Syzygiumn aromaticum* L against *Dermestes maculatus* DeGeer 1774 (Coleoptera: Dermestidae) infesting smoke-dried *Clarias gariepinus* Burchell 1822. *Journal of Applied Entomologist*, *2*(2), 01-05.

Suleiman, M., Sani, I., Yusuf, A. M., and Abdullahi, B. K. (2019). Entomocidal activity of some plant extracts against Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae). *African Journal of Agricultural Research*, *14*(34), 1862-1869.

Szczepanik, M., Zawitowska, B., and Szumny, A. (2012). Insecticidal activities of *Thymus vulgaris* essential oil and its components (thymol and carvacrol) against larvae of lesser mealworm, *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae). *Allelopathy Journal*, 30(1), 129-142.

Trivedi, A., Nayak, N., and Kumar, J. (2017). Fumigant toxicity study of different essential oils against stored grain pest *Callosobruchus chinensis*. *Journal of Pharmacognosy and Phytochemistry*, 6(4), 1708-1711.

V. Jumbo, L. O., Haddi, K., Faroni, L. R. D., Heleno, F. F., Pinto, F. G., and Oliveira, E. E. (2018). Toxicity to, oviposition and population growth impairments of *Callosobruchus maculatus* exposed to clove and cinnamon essential oils. PloS one, 13(11), e0207618.

Wilane, D., Gueye, S., Gueye-N'Diaye, A., and Seck, D. Fumigation toxicity of three plant essential oils against Dermestes spp. larvae,(Coleoptera Dermestidae), a major pest of dried fish in the Tropics

PRESERVATIVE ACTIVITY AND ACUTE TOXICITY (LD50) OF *MORINGA OLEIFERA* SEED OIL ON BOILED WHITE RICE

*** Raubilu, I. A.**
[1]Usman A.D.
[2] Abdul N.A[3]
Aisha A.S.[3]
[1]Department of Basic and Applied science, Hassan Usman Katsina Polytechnic, Katsina, Nigeria
[2]Department of Microbiology, Bayero University Kano, Kano, Nigeria
[3]Department of Microbiology, Umaru Musa Yar'adua University, Katsina, Nigeria

ABSTRACT
Plants, either as traditional preparations or pure active principles, have always been among the common sources of medicines and preservation. Over 5000 plants are known to be used for medicinal and preservative purposes in Africa, but only a few have been studied. The objectives of the study are detection of phytochemical constituents and evaluation of antimicrobial, preservative and acute toxicity properties of *Moringa oleifera* seed oil on boiled white rice using
Standard procedures. The results of the phytochemical screening revealed presence of Alkaloids, saponins, tannins and flavonoids. Antibacterial activity of the *Moringa oleifera* seedoil showed highest zone of inhibition diameter against *Escherichia coli* (16.25mm) while antifungal activity was pronounced against *Saccharomyces spp* (24.20mm) using 100% oil. Results of sanitizing activity of the 100% oil revealed 4.37log count (cfu/g) reduction (bacteria), 4.70log count (cfu/g) reduction (fungi) for boiled white rice after96 hours of storage. Results of organoleptic assessment and general acceptability showed that treatment with 50% of oil revealed 6.3±0.67(40) likeness by judges as compared to 3.7±0.82(10) likeness for control (untreated) after 48hrs hours (2 days) for boiled white rice. The LD_{50} *of Moringa oleifera* seed oil *was estimated to be more than 5000 mg/kg.* Findings from this study showed that *Moringa oleifera* seed oil possess antimicrobial, preservative activities on boiled white rice. These results suggest that thepetroleum ether *Moringa oleifera* seed oil is rich in phytochemicals, and may be considered relatively safe at the tested doses. The oil is a candidate for food preservation.
Keywords: Preservative activity; acute toxicity; *Moringa oleifera* seed oil; boiled white rice.

INTRODUCTION
Preservation of food has been used since ancient times. The preservation process will restrain the development of microbial such as bacteria and fungi (*Sharif et al.*,2017). In this light, the aims of food preservations include, first, maintaining food taste, texture, flavour, quality and nutritional value, second, to reduce the wastage of excess food, third, to maintain a product's accessibility for a longer time, even in places it is not being produced, fourth, to preserve the food materials during transportation, and finally, to ease the handling of food materials (*Sharif et al.*,2017).
Some of these preservation methods use additional food preservatives which can be classified into artificial and natural preservatives (*Sharif et al.*,2017). Generally, consumers are aware of the short and long term effects of using artificial preservatives to their health. Thus, the demands of using natural preservatives in food are highly increasing (*Sharif et al.*,2017).

Plants, either as traditional preparations or pure active principles, have always been among the common sources of medicines and preservation. Over 5000 plants are known to be used for medicinal and preservativepurposes in Africa, but only a few have been studied [Adebayo*et al.*, 2010]. A great number of Nigerian higher plants are traditionally noted for their medicinal properties, but regrettably only few have so far been studied for their active constituents. The use of herbal remedies can be attributed to their perceived efficacy and the fact that they are a cheap source of medicines [Ogbonnia et al., 2010].

There is an assumption that the use of plants intraditional medical practice for treatment ofvarious ailments is harmless and safe because they are derived from natural sources [Alli et al., 2015]. This assumption is based on the common belief that herbs are by nature safer and gentler than orthodox drugs and plant-based medicine have been used in the treatment of diseases over many centuries [Newman and Cragg 2007].

Moringa oleifera on the other handis a woody tree mainly distributed in the tropical and subtropical regions of Asia, Africa (including Nigeria) and the Middle East (Raubilu et al., 2019). Moringa oleifera is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal usage with high nutritional contents. Different tissues of this plant contain a profile of important ant agents (Bukar et al., 2010)

MO seed oil is edible and reported to be a rich source of monounsaturated fatty acid, particularly oleic acid (C18:1) (71-74% of total fatty acids) (Ruttarattanamongkol *et al.*,2014). High-oleic acid oils have been proved to be more resistant toward oxidation (Anwar *et al.*, 2003). Our previous study suggested that the high oleic acid content of the *MO*oil coupled with its low peroxide value and acidity may qualify the *MO* oil as a natural derived antioxidant in food and cosmetic industries (Ruttarattanamongkol *et al.*, 2014).

Investigation of phytochemicals and determination of efficacy and safety of herbal remedies is necessary as many people use them for self – medication and preservations. It should, therefore, be emphasized that the traditional use of any plant for medicinal or preservative purposes warrants the safety of such plant. Plants in folk medicine should, therefore, be evaluated for safety or toxicity and necessary recommendations be made on their use. Therefore, the current study was aimed at investigating the preservative, phytochemical composition, Antimicrobial and determining the acute (LD50) toxicity of the petroleum ether seedoil extract of *M.oleifera.*

MATERIALS AND METHOD

Collection and Authentication of Plant Materials

The *Moringa oleifera* seed was collected from the Central market around Katsina Local Government area of Katsina State, Nigeria in a sterile polyethene bag. It was identified and authenticated at the Herbarium of the Department of Plant Biology, Umaru Musa Yar'adua University, Katsina (UMYUK) and Confirmation of taxonomic identity of the plants was achieved by comparison with voucher specimens kept at the Herbarium of the Department of Biological Sciences, UMYUK. All reagents and chemicals used were from E. Merck or Sigma-Aldrich.

Extraction of essential oil

Oil extraction was done by adding petroleum ether to the seed powder. To separate the oil from the seeds, the electro thermal Soxhlet was used and hexane was passed in to the apparatus to extract the oil. The hexane was then evaporated leaving the concentrated oil, which was then stored at room temperature. The extracted seed oil was then kept for further analysis (Bichi and Shehu 2018).

Phytochemical Screening

Phytochemical analysis for qualitative detection of alkaloids, flavonoids, Phenolic compounds, reducing sugar, tannins and saponins was performed on the extracts as described Bukar et al., 2010.

SOURCES OF MICROORGANISMS

Test Organisms (Bacteria); *S.aureus, E.faecalis, S.typhi, S.flexneri, P.aeruginosa,* and *E.coli* and Fungi: *Saccharomyces spp* to be used for the study were isolated from food samples commonly consumed in katsina local government, which included rice and beans dish, cold tiger nut drink (Kunun Aya), Tomato and Raw meat. This was followed by homogenizing and streaking food samples on appropriate media for isolation. Further microscopic and biochemical characterization of the organisms were carried out according to methods described by Cheesebrough (2002).

Standardization of Bacteria Inocula

The Inocula of the test organisms were standardized using 0.5 McFarland's turbidity standard. Approximately 99.5ml of 1% $BaCl_2$ were added to 0.5ml of 1% H_2SO_4 in order to obtain 100ml of $BaSO_4$ which corresponded to 0.5 McFarland's turbidity standard equivalent to 1.0 X 10^8 cfu/ml populations for bacterial isolates (Salisu *et al.,* 2017). Bacterial colonies from the 18hours overnight cultures were used to make direct suspension of each bacterial isolate in separate test tubes containing 1ml of sterile physiological saline. The suspensions were adjusted to match the 0.5 McFarland turbidity standards, using saline and a vortex mixer (Salisu et al., 2017).

3.6.6 Standardization of Fungal Inocula

A loopful of fungal spores from an overgrown plate was taken and shaken thoroughly in 10ml of 20% Tween 80 solution to arrive at 6.0 x 10^5 cfu/ml of the spore suspension (Salisu et al., 2017)

Antimicrobial Disc Preparation

The antimicrobial activity of the extract was tested using agar well diffusion method adopted from CILS (1998). Appropriate culture media were prepared for the bacterial and fungal isolate to be tested. The plates were separately inoculated with the suspension of the standardized cultures using sterile swabs. Two wells of 6mm diameter were made in each plate using 6mm sterile cork borer. The wells were filled with 0.1ml of diluted concentrations (100%, 75%, 50% and 25%) of the oils with the aid of sterile pipettes per well. Sterile distilled water and 10µg/ml of Ciprofloxacin were used in separate plates for each organism to serve as negative and positive controls respectively (Salisu *et al.,* 2017).

Diameters of the zones of inhibition will be measured with a transparent ruler and the result will be recorded in millimeters after incubating the plates at 37^0C for 24 hours. The plates will be replicated in duplicates and the means and standard errors of the zones of inhibitions for each organism at each concentration of each extract will be calculated and recorded accordingly as described by *Salisu*Baha'uddeen *et al.* (2017b).

Evaluation of sanitizing activity of *M. seed oil*on Boiled white rice

Commercially prepared boiled white rice was purchased from a local vendor at central market, katsina local government, Katsina state.

Five (5) different concentrations of the essential oils will be applied on 5g of the rice, each in different sterile plastic container, arriving at a concentration of 0%,(w/v) (control), , 50%(w/v), 75%(w/v) and 100%(w/v).

Enumeration of Aerobic Plate Count and fungal count was carried out in duplicate determinations. The mean value was taken, and the room temperature was monitored throughout the period of experiment as described by Bukar (2012).

Sensory Evaluation

Commercially prepared boiled white rice were purchased from a local vendor at central market, katsina local government in Katsina state.

Five (5) different concentrations of the essential oils will be applied on 5g of the rice and bread, each in different sterile plastic container, arriving at a concentration of 0%,(w/v) (control), 25%(w/v), 50%(w/v) and 75%(w/v). Number of days taken for each treatment to spoil will be recorded. Organoleptic parameters namely, taste, texture, odor, and color will be assessed by a panel of ten (10) judges. The score were graded on a Hedonic scale and after 9hrs of storage, the treatment most preferred by the judges will be determined (Bukar, 2012).

Lethal Mean Dose (LD50) Determination

The limit test procedure described by Organization for Economic Cooperation and Development (OECD) guidelines was adopted [OECD, 2001)]. Five (5) rats of either sex, selected by random sampling, were used for the study. The animals were fasted overnight, providing only water, after which the aqueous stem bark extract, was administered orally at a dose level of 5000 mg/kg body weight to each rat at 48 hours interval. Each rat was kept under observation for the first 4 hours and within the period of the 48 hour. Behavioral changes (weakness, difficulty in movement, sedation, hyperactivity, hair loss, reduced response to sound, loss of appetite, grooming), body weight, and mortality were observed for a period of 14 days.

3.13 Statistical Analysis

Data generated from the sanitizing activity tests and the scores generated based on the assessment of judges (from the preservative experiment) using hedonic scale, were statistically analyzed using analysis of variance (ANOVA) at 5% probability level using the software package developed by Microsoft corporation. Where significant difference was observed, the means separation was carried out using least significant difference (LSD)

RESULTS AND DISCUSSION

Table 1 show that *M. oleifera* essential oil had a yield of 190ml per 700g (27.1%) of the seed. The result of moringa seed oil showed a high percentage yield which is in accordance with researches of Eman and Muhamad (2016). Moreoverthe results obtained here are in agreement with [Dalia et al., 2007; Abdulkarim et al.,2007]. The results from table 1 revealed the taste of the various extracts which is very important revelation as the oil would be used in sanitizing and preservative experiments. The results strength of their taste, colour and odour might infer on the sensory properties of the foods, which could determine their acceptability or otherwise. Phytochemical analysis of *M.oleifera* oil reveal the presence of secondary metabolites(Table 2). The result reveals the presence of alkaloids, saponins, tannins, flavonoids. The result do not corroborates with the findings of Abdulrasheedet al., (2015). The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Phenolic compounds are known to possess different pharmacological activities, among which antioxidant and antimicrobial effects have recently received more intention.

Table 1: Physical Characteristics of the Extracts and Oil

Physical Parameters	Weight of seed/leave (g)	Weigh of oil(/ml)	Colour of extracts	Texture	Taste	Percentage yield
M. seed oil	700	190	Pale yellow	Semi viscous	Mild nutty flavour	27.1

Table 2:**Phytochemical Constituents of extracts of *M.oleifera* and essential oil**

Phytochemicals	**Alkaloids**	**Saponins**	**Tannins**	**flavonoids**	**Phenolic compounds**	**Reducing sugars**
M. seed oil	**+**	**+**	**+**	**+**	**-**	**-**

KEY: + Present, **- A**bsence

Table 3 presents the results of the antimicrobial activityof *M.oleifera* seedoil (MLSO)against bacterial and fungal isolates.The Result of the antibacterial activity of MLSO revealed that only *S.typhi, S.flexneri, P.aeruginosa*, and *E.coli* were active at the different concentrations of the extracts tested. However, MLSO do not revealed any antimicrobial activity against *S.aureus* and *E.faecalis.* The result of MLSO against *E.coli* obtain corroborate with findings of Bichi and Shehu (2018)who reported the antibacterial activity of MLSO on *E.coli*. The result of MLSO against *Salmonella typhimurium*, and *Pseudomonas aeruginosa* obtain agrees with the findings of Khanitta and Angelika (2015). However, the result of MLSO against Staphylococcus aureusobtain do not corroborate with findings of Khanitta and Angelika (2015) who reported the antibacterial activity of MLSO against Staphylococcus aureus.The result obtain from MLSO shows antifungal against *Saccharomyces spp.* The results of the present study shoes that most of the organic solvent extracts of Moringa oleifera seed oil showed significant activity against the tested fungal strain with the highest concentration of the essential oil having the highest zone of inhibition.There was no statistical significant difference (P>0.05) among the organisms tested at highest concentration.

Table 3: Zone Diameter of Inhibition (mm) of *Moringa* seed oil against Isolates

	MLSO Concentration in %				**MIC**	**MBC /MFC**
Organisms	100	75	50	25		
Staphylococcus aureus	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	-	-
Enterococcus faecalis	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	-	-
Salmonella typhi	13.75± 0.35	8.25± 0.35	7.15± 0.92	0.00± 0.00	50%	>50%
Shigella flexneri	16.10± 0.14	9.35± 0.21	8.25± 0.35	7.25± 0.35	25%	50%
Pseudomonas aeruginosa	13.25± 1.77	8.25± 1.76	7.13± 0.18	0.00± 0.00	50%	50%
Escherichia coli	16.25± 1.77	11.13± 1.59	7.5± 0.71	7.25± 0.35	10%	50%
Saccharomyces Spp	24.20± 1.13	21.85± 0.92	18.65± 1.20	14.8± 0.42	10%	10%

KEY: MLSO:*Moringa oleifera* Seed oil petroleum ether extracts, Values are mean ± SE and each value is mean of two determinations, **>concentration:** Growth not inhibited, **Exact concentration:** Growth inhibited, **MIC:** Minimum Inhibitory Concentration**, MBC:**Minimum bactericidal Concentration, **MFC**: Minimum Fungicidal Concentration.

Figures 1 and 2 showed the result of the sanitizing activity of *Moringa* seed oilon boiled white rice.The result obtain from the sanitizing activity of Rice using Moringa seed oil indicates the reduction of the APC and FC count after 48hrs and 72hrs of storage respectively. The sanitizing activity of boiled rice shows high FC 4.95 and APC 4.88log counts at 0hrs. This could be due to poor processing as well as pre and post production contamination (Kawo and Abdulmumin 2009; Bukar et al., 2009). The sanitizing effect varies between concentrations with the highes concentration (100%) having the must sanitizing effect. This also corroborate with the findings of Sofowora (1982) and Yushau *et al.*, (2009), who reported that plants extracts used in ethno medicine show antimicrobial activity in a dose dependent manner.

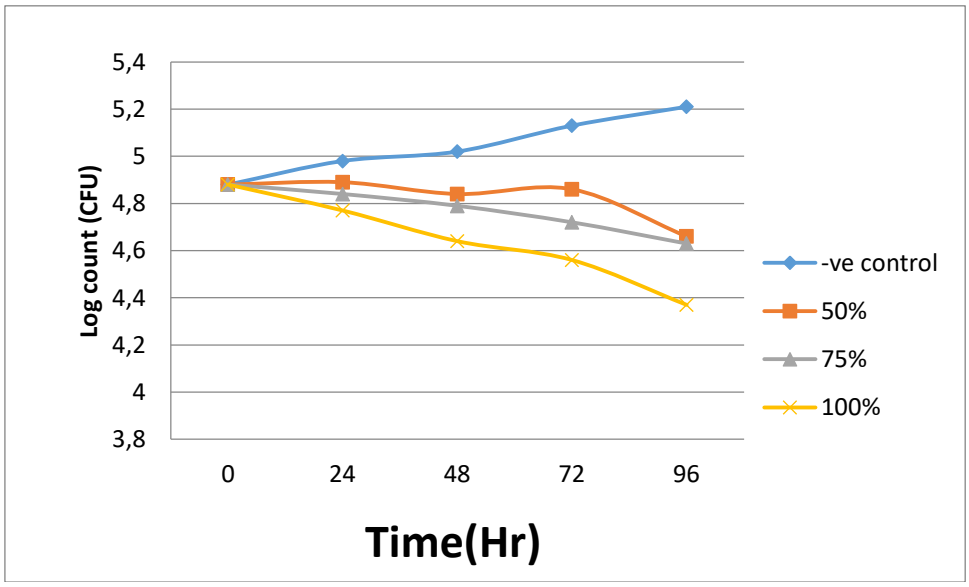


Fig 1:Sanitizing activity (bacterial count reduction) by *M.oleifera* seed oilon boiled rice stored for 96hrs

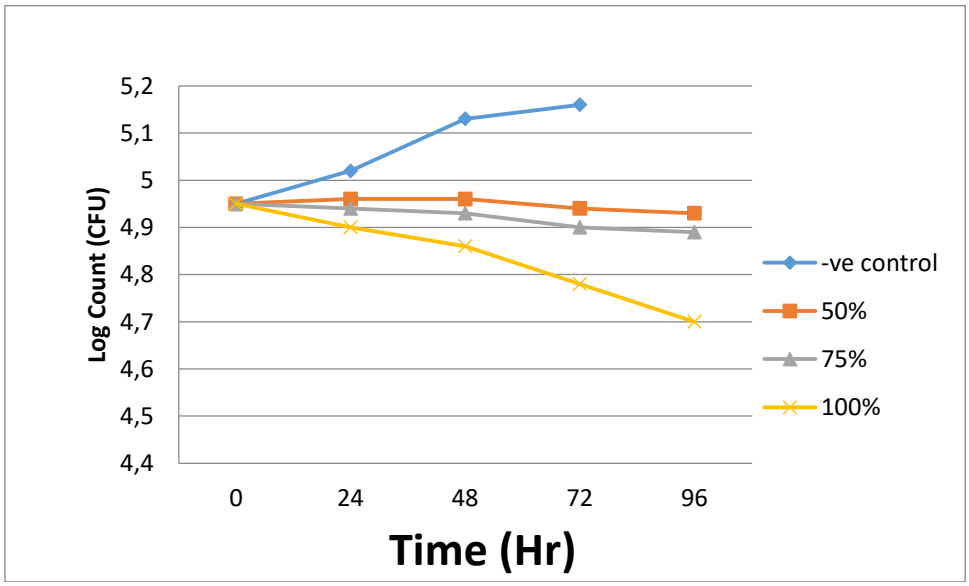


Fig 1:Sanitizing activity (Fungal count reduction) by *M.oleifera* seed oilon boiled rice stored for 96hrs

The organoleptic assessment showed of boiled white rice is presented in Table 4. The consumer acceptability of the processed boiled white rice was evaluated by the selection of panelist. The nine point hedonic rating test was used to determine the acceptability as described by Bukar (2012). The sensory profile of boiled white rice was evaluated by 10 panelists, using simplified quantitative descriptive analysis (Tomlins *et al.,* 2012). The panel consisted of students and Staffs of the Umaru Musa Yaradua University.

Previous studies such as research conducted by Kaneko et al., (1999) have shown that the nutritional value of rice is provided by its content such as protein, carbohydrate etc and a host of vitamins and nutrients. From the result, the organoleptic assessment showed that the judges rejected the untreated boiled rice after 48hrs of storage with a percentage likeness of 40% compared to 100% at 0hrs. Treatment B recorded the highest number of days 72hrs before which the food is rejected with percentage likeness of 30% compared to its 90% at 0hrs. According to the judges, treatment C and D were rejected after 48hrs (2days) of storage. Based on the findings, treatment C was found to be most effective.

General acceptability (percentage likeness) of preserved boiled rice by judges (0-9)

		Treatments		
Storage(hour)	**A**	**B**	**C**	**D**
0	9.7±0.48(100)	9.4±0.70(90)	9.0 ±0.81(80)	8.5±0.52(80)
24	7.4±0.52(70)	7.5±1.43(70)	7.1±1.10(50)	7±0.67(60)
48	3.7±0.82(10)	6.3±0.67(40)	4.3±0.95	4.6±0.52
72	1.8±0.63(0)	5.8±0.42(30)	4.7±0.48(20)	4.8±0.42(20)
96	1.0±0.00(0)	1.5±0.5(10)	1.5±0.7(0)	1.6±0.70(10)

A= Untreated, B= Treated with 50% seed oil, C= Treated with 75% seedoil, D= Treated with 100% seed oil, Values are mean ± SE and each value is mean of ten determinations. Scale: 9= Like extremely, 8= like very much, 7= like moderately, 6= like slightly, 5= neither like nor dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much and 1= Dislike extremely

The result of acute toxicity of MLSO is presented in table 5.The oral acute administration of 5000mg/kg body weight of MLSO resulted in no mortality. This suggests that the LD50 is greater than 5000mg/kg and can be classified as practically non-toxic using the Ukwuani *et al*., (2010) classification of range of LD50. However, behavioral changes observed in this study may be attributed to secondary metabolite content of the extract such as Saponins (Abubakar*et al.,* (2008)) since its toxicity effects includes; fatigue, malaise, confusion and anorexia(Ukwuani *et al*., (2010)). LD50 greater than 5g/kg body weight is considered safe by the recommendations of World Health Organization (WHO) and Organization for Economic and Cultural Development (OECD) guideline respectively

Table 5: Acute Oral Toxicity of *M.oleifera* Seedoil

Group	**Extracts**	**Dose (mg/kgbw)**	**Mortality**	**Behavioral changes**
A	Seed oil	5000mg/kg	0/5	Mild restlessness, erection of hair coat, Increased respiratory rate in the first 5 minutes, confusion, scratching of their nostrils and head, anorexia, Sensitive to slight sound, moving around restlessly and whipping of their mouths

Conclusion

In Conclusion, this study has provided evidence on the antimicrobial, sanitizing and preservative properties MLSO due to the presence of phytochemicals such as Saponins, Tannins, and Alkaloids. The antimicrobial activity clearly shows a broad zone of inhibition of MLSO against the tested isolates. Furthermore, the result from the sanitizing and preservative activity of the oil indicate their ability to reduce microbial count from the foods, as well as increase the shelf life. The result from the acute toxicity also reveal that the essential oil produce no mortality and can be classified as non-toxic which makes it a candidate for food preservation

REFERENCE

Abdulkarim,S. M. K. Long, O. M. Lai, S. K. S. Muhammad and H. M. Ghazali, "Frying Quality and Stability of High-Oleic Moringa oleifera Seed Oil in Comparison with Other Vegetable Oils," Food Chemistry, Vol. 105, No. 4, 2007, pp. 1382-1389. Doi:10.1016/j.foodchem.2007.05.013

Abdulrasheed, M., Ibrahim, I.H., Mubarak, M.A. and Umar, F.A. (2015). Comparison of antimicrobial activity of seed oil of garlic andmoringa oleifera against some food-borne microorganisms. Bayero Journal of Pure and Applied Sciences, 8(2): 196 – 201. Http://dx.doi.org/10.4314/bajopas.v8i2.32

Adebayo AH, Abolaji AO, Opata TK, Adegbenro KI. Effects of ethanolic leaf extract of *Chrysophyllum albidum* G. on biochemical and haematological parameters of albino Wistar rats. Afr. J. Biotechnol. 2010;9(14):2145-2150.

Alli LA, Adesokan AA, Salawu OA, Akanji MA. Toxicological studies of aqueous extract of *Acacia nilotica* root. Interdisciplinary Toxicology. 2015;8(1):48–54.

Anwar, F., &Bhanger M.I. (2003): Analitical characterization of Moringa oleifera seed oil grown in temperate regions of Pakistan. *Journal ofAgricultural and Food Chemistry*, 51, 6558-6563.

Bichi, M. H., and Shehu, U. H.(2018).Antimicrobial properties of *moringa oleifera* seed oil:*International journal of research in earth & environmental sciences*. Vol. 13, no.1.

Bukar, A. (2012): preservative properties of plant extracts and oils on some foods. Lambert academic Publsihing. AV Akademikerverlag GmbH and Co.KG.

Bukar, A., Uba, A. And Oyeyi, T.I. (2010): Antimicrobial profile of moringa oleifera lam. Extracts against some food – borne microorganisms. Bayero Journal of Pure and Applied Sciences, 3(1): 43 – 48.

Cheesbrough, M. (2000). District laboratory practice in tropical countries, part 2. Cambridge university press. Unitted kingdom. Pp 38-43

CILS and Antimicrobial SusceptibilityTesting. (1998). Performance Standards for Antimicrobial Susceptibility Testing. In Blood Safety and Clinical Technology Guidelines on Standard Operating Procedures for Microbiology. Eight Informational Supplement. M100- S8, 18 (1), CILS Pennsylvania, USA.

Dalia I, Sánchez M, Jaime L, José A N, Gabriela S Mora, Julia L amd Perfecto P 2015 *Food Chemistry* 187 pp 53–57

EmanN. A., and MuhamadK. N. S. (2016). Comparison of Moringa Oleifera seeds oil characterization produced chemically and mechanically. International Conference on Chemical Engineering and Bioprocess Engineering IOP Publishing IOP Conf. Series: Earth and Environmental Science 36 (2016) 012063 doi:10.1088/1755-1315/36/1/012063

Kaneko, K.I., Hayashidani, H., Ohtomo, Y., Kosuge, J., Kato, M., Takahashi, K., Yasuo, S. And Ogawa, M. (1999): Bacterial contamination of ready to eat foods and fresh products in retail shops and food factories. Journal of food protection. 62(6): 644-647

Kawo, A.H and Abdulmumin, F.N. (2009): Microbiological quality of pre-packed sweet sold in metropolitan Kano, Nigeria. Bayero journal of pure and Applied Sciences 2(1):154-159.

Khanitta R., and Angelika P. (2015): Antimicrobial activities of Moringa oleifera seed and seed oil residue and oxidative stability of its cold pressed oil compared with extra virgin olive oil**.** Songklanakarin Journal of Science and Technology. 37 (5): 587-594.

Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. J Nat Prod. 2007;70:461–477.

OECD (2001)-. Adopted by the council on 17 December. Organization for Economic Co-operation and Development (OECD) guideline for the testing of chemicals, Acute oral toxicity –Acute toxic class method.[Internet]Available from: http://iccvam.niehs.nih.gov/SuppDocs/ FedDocs/OECD/OECD_GL423.pdf.

Ogbonnia SO, Mbaka GO, Anyika EN, Ladiju O, Igbokwe HN, Emordi JE, Nwakakwa N. Evaluation of anti-diabetics and cardiovascular effects of *Parinaricuratellifolia* seed extract and *Anthoclistavogelli* root extract individually and combined on postprandial and alloxaninduced diabetic albino rats. Br. J. Med. Res. 2010;1(3):146-162.

Raubilu, I.A., Isah, U., Ahmad M.A. (2019):Antimicrobial activity ofmoringa oleifera: a short reviewbayero Journal of Pure and Applied Sciences, *Special Conference Edition* 12(1):ISSN 2006 – 6996http://dx.doi.org/10.4314/bajopas.v12i1.1.

Ruttarattanamongkol, K., and Petrasch, A. (2015): Antimicrobial activities of *Moringa oleifera* seed and seed oil residue and oxidative stability of its cold pressed oil compared with extra virgin olive oil.***Songklanakarin Journal of Science and Technology***. V 37 (5), 587-594.

Salisu D. B., Magashi, A. M., Abdulkadir, B. (2017b). Phytochemical Determination and *In Vitro* Antimicrobial Activity of Crude EthanolicExtractof Stem Bark of *Boswelliadalzielii. International Journal of Science and Research (IJSR).* Volume 6 Issue 12.

Salisu, B., Magashi, A. M., & Mohammed, A. (2017). Determination of Phytochemicals and Antimicrobial Activity of Aqueous Stem Bark Extract of Boswelliadalzielii against Some Common Pathogenic Microorganisms. *UMYU Journal of MicrobiologyResearch*, *2*(1), 238–246. Retrieved from www.umyu.edu.ng.

Sofowora, E.A. (1982): Medicinal and |Traditional Medicine in in Africa. Wiley and Sons, London. Pp 249.

Tomlins, K., Owori, C., Bechoff, A., Menya, G. And Westby, A. (2012): Relationship among the carotenoid content dry matter content and sensory attributes of sweet potato. Food Chem. 131:14-21. Https://doi.org/10.1016/j.foodchem.2011.07.072.

Yusha'u, M., Balarabe, A.I. and Bukar, A. (2008): prevalence and sensitivity of Enterobacterial isolates of Urinary Traact to Acalypha wilkesiana Extracts. Biological and Environmental Sciences Journal for the tropics 5(3):72-76.

SYNTHESIS OF METAKAOLIN GEOPOLYMERS BY REPLACEMENT OF SODIUM SILICATE ACTIVATOR WITH GUINEA CORN HUSK ASH

[1]Ahmad, Z.M.
[2]Saleh, M.S.
[3]Salisu, A.
[4]Ilu, M.B.
[1,2,3,4] Department of Pure and Industrial Chemistry Umaru Musa Yar'dua University, Katsina state, PMB 2218, Nigeria

ABSTRACT

The synthesis of geopolymers for the replacement of Portland cement has been explored due to the low CO_2 emission that is involved which can attribute global warming. In this research syntheses of inorganic polymers using metakaolin as starting materials and sodium silicate, (Na_2SiO_3) sodium hydroxide, (NaOH) and guinea corn husk ash, (GCHA) as activator was carried out. The activator 10% were mixed with the metakaolin for 5 minutes, the water was added, the resulted pastes were casted in to 50×50×50mm mold for compressive strength test and 140×40×40mm for the flexural strength test. The geopolymers were cured at 60^0C for 20 hours. The casted samples were subjected for mechanical strength test after 7 and 28days of curing at ambient temperature. This procedure was repeated by total replacement of sodium silicate with guinea corn husk ash. The geopolymers cured after 28days were analyzed for water absorption test, acid resistance test using H_2SO_4, FTIR, XRD, SEM and TGA. The microstructure, mineralogy and thermal stability confirmed the formation of geopolymers with sodium aluminosilicate hydrates (N-A-S-H), the water absorption and chemical stability described that both geopolymers were slightly dense in structure and resistant to acidic attack. The highest compressive and flexural strength cured for 28 days were 17.06 N/mm^2, 2.31 N/mm^2 and 10.86 N/mm^2 and 0.91 N/mm^2 for Na_2SiO_3 geopolymers (SSG) and GCHA geopolymers (GCHG) respectively. This study revealed that both the geopolymers can be used as eco-friendly materials for the substitute of ordinary Portland cement without much effect to the environment.

Keywords: Guinea corn husk ash, Flexural strength, Microstructure, Mineralogy, Environment.

EVALUATION OF ELEMENTAL SELENIUM CONCENTRATIONS ANALYZED IN DRINKING WATER SAMPLES CONSUMED IN KASTAMONU PROVINCE, TURKEY

Aybaba HANÇERLIOĞULLARI
Şeref TURHAN
Aslı KURNAZ
Department of Physics, Faculty of Science and Letters, Kastamonu University, Kastamonu, Turkey

ABSTRACT
Selenium (Se) is an essential and also toxic element to living things. Se carries the narrowest range between nutritional deficiency (< 40 µg/d) and toxicity (> 400 µg/d) relative to daily intake. Elemental Se, in very variable concentrations, is found primarily in ground and surface waters. In this study, Se concentrations analyzed by inductively coupled plasma optical emission spectroscopy in 64 drinking water samples (30 tap, 13 bottled spring, and 21 bottled mineral water samples) consumed in Kastamonu province were evaluated. All Se concentrations analyzed in bottled spring waters remained below the detection limit (DL) of 0.5 µg/L, while Se concentrations analyzed in only four tap water samples varied from 0.8 to 7.6 µg/L. Se concentrations analyzed in 21 bottled plain and fruit-flavored bottled mineral waters varied from < DL to 217.6 µg/L with an average of 143 µg/L, which is higher than the maximum permissible concentration of 10 µg/L set by national and international organizations.
KEYWORDS: Selenium, tap water, bottled spring water, bottled mineral water, Kastamonu, Turkey

1. Introduction
Water is necessary for a sustainable life. There are various types of drinking water (tap water, bottled water, etc.) available for human consumption from different water sources such as surface and groundwater. However, these drinking water sources are mostly polluted by both natural and anthropogenic activities, and this pollution remains one of the challenging global problems [1]. For the quality and safety of drinking water, it should not contain pathogenic organisms, toxic elements, organic substances, an overdose of minerals, turbidity, color, taste, and odor. For this reason, various national and international organizations have established regulations and standards and prepared guidelines to continuously monitor and control the quality and safety of water for human consumption.
Selenium (Se) is a common and naturally occurring trace element [2]. Due to its applications (electronic components, glass additives, metal alloys, etc.) and its impact on human and animal health, research on selenium has attracted attention and environmental pollution issues have come to the fore [3]. Selenium emerges as a serious health hazard due to its presence in groundwater used as a source of drinking water in various parts of the world. The selenium concentration in such contaminated water is sometimes as high as 400-700 µg/L [4]. Se pollution in natural waters can be of anthropogenic or geogenic origin [2]. Both geogenic and anthropogenic activities govern Se in the environment. Environmental biogeochemical conditions control the bioavailability and nature of selenium species [5]. Geogenic pollution of groundwater is referred to as the natural occurrence of high concentrations of certain elements in groundwater with adverse health effects [5]. Geogenic Se contamination is a concern for toxicity from drinking water. Anthropogenically, coal mining and the use of Se-rich fossils greatly contribute to Se pollution in the environment [5]. Se can occur in

inorganic, organic, and amino acid compounds in biological systems as well as in the environment [4]. Inorganic Se compounds such as selenate (SeO_4^{2-}), selenite (SeO_3^{2-}), selenite (Se^{2-}), and elemental Selenium (Se^0) is mainly found in surface and groundwater. Organic species are generally common in terrain and plants such as methylselenides, methylselenol, dimethyldiselenide, dimethylselenium sulfide, and selenoamino acids [4].

Se is now known to be an essential trace element for both human and animal health but can be toxic at high levels of intake [3] Se is required as a micronutrient for several physiological and biochemical processes mainly as a component of selenoproteins or aminoacids such as selenomethionine and selenocysteine [3]. Se deficiency and Se toxicity are linked with serious diseases [3]. The consequences of Se deficiency include Kashin-Beck disease, an endemic osteoarthritis disease that disrupts bone structure [3]. However, if the daily intake rates are too high, human health may be adversely affected. Recent epidemiological studies have shown that overexposure to Se, both to its less toxic organic forms and more toxic inorganic species, can be detrimental to human health [6]. Chronic exposure to high Se levels results in selenosis, which can cause hair loss, nail brittleness, skin rashes, neurological damage, gastrointestinal problems, and even liver cirrhosis and death [3]. Se has the narrowest range in terms of daily intake between nutritional deficiency and toxicity [3,7]. The recommended dietary intake of Se varied from 40 to 400 μg/d [3].

Until now, many studies were conducted to determine the concentration of Se in drinking water, as insufficient or excessive intake of Se and its corresponding effects on human health are only narrowly separated [2-23]. However, there are several studies on the analysis of Se in drinking water for human consumption in Turkey [24-28] but there is no study related to the analysis of elemental Se concentrations in drinking waters consumed in Kastamonu province. Kastamonu is an important nature tourism destination due to the nature of the landscape and the coast [29]. The population of Kastamonu province as per the 2021 census is 375,582 of which 152,541 live in the urban center. In this study, the concentrations of Se in sixty-four drinking water samples (thirty tap waters, thirteen bottled spring waters, and twenty-one bottled mineral waters) collected from different sampling locations chosen to reflect the distribution of population and purchased from markets in Kastamonu were analyzed by using an inductively coupled plasma optical emission spectrometry (ICP-OES).

2. Materials and method

Thirty tap water (TP) samples were collected from the different houses located in all parts of the Kastamonu province (Fig. 1). Thirteen bottled spring water (BSW) and twenty-one bottled plain and fruit-flavored mineral water (BMW) samples of popular commercially brands were bought from markets in Kastamonu. Each water sample was opened in the laboratory. For the selenium analysis process, each drinking water sample was filtered from microfilters so that no particles were left, and then TP and BSW samples were taken into the analysis process without any process. However, BMW samples were digested with a microwave digestion system (CEM MARS 6, USA) equipped with temperature and pressure control to 200 °C and 45 bar.

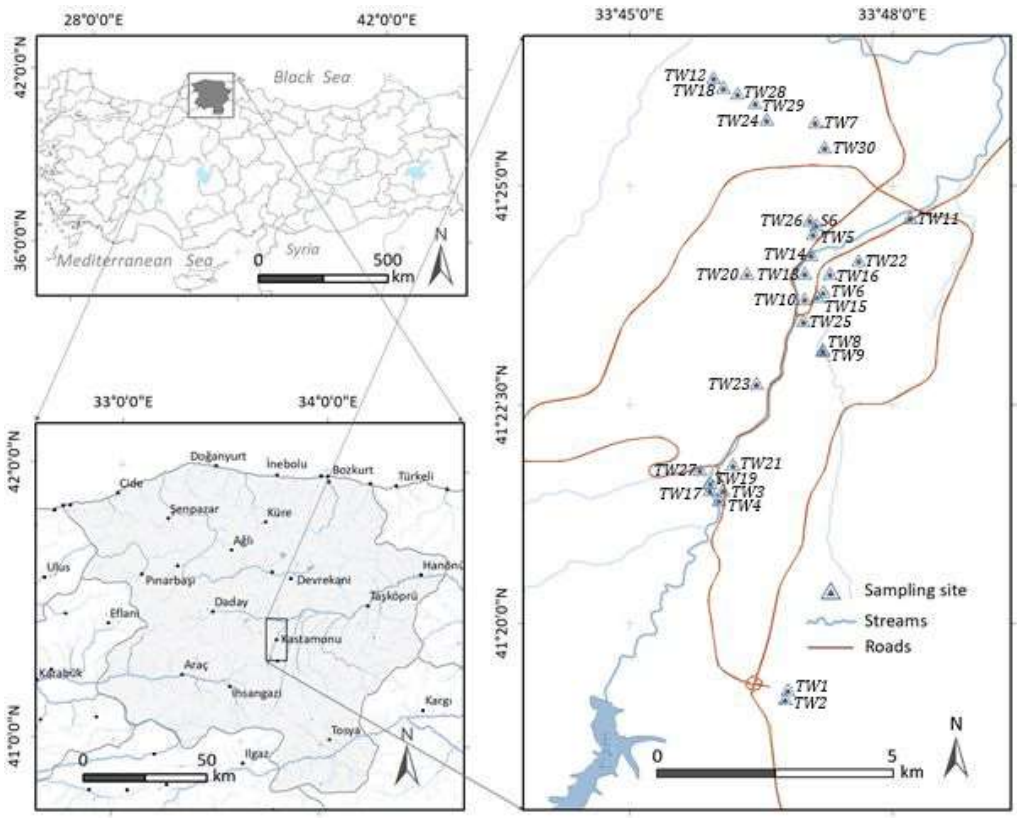


Figure 1. Locations of tap water samples [29]

The Se concentrations were analyzed by the Spectroblue ICP–OES system equipped with Spectro's proprietary ICP Analyzer Pro software making it easy to take full advantage of the instrument's simplified operation and analytical capabilities. Calibration solutions of the linear range of 0.01– 0.1 mg/L were prepared by diluting the certified standard ICP TraceCert mix solutions (10 mg/L) purchased from Sigma–Aldrich. Calibration of the ICP–OES system was carried out at the beginning of the measurements and the correlation coefficients were higher than 0.999 for all analytes. The detection limit of Se was found as 0.5 µg/L.

3. Results and discussion

The concentrations of elemental Se analyzed in the drinking water samples and the maximum allowable concentrations set by the Turkish standard (TS) [30], Turkish legislation (TL) [31] European Communities directive (EC D) [320], and EPA standards [33] is given in Table 1. Also, the comparison of the average Se concentration analyzed in drinking water samples with the maximum allowable concentrations recommended by TS, TL, EC D, and EPA is shown in Fig. 2. Se concentrations analyzed in a total of thirteen bottled spring water samples were found below the detection limit of 0.5 µg/L.

It can be seen from Table 1 that the Se concentrations analyzed in only four tap water samples (TW-22, TW-23, TW-24, and TW-31) were found above the detection limit. The concentrations of Se analyzed in four TW samples varied from 0.8 to 7.6 µg/L with an average of 3.6 µg/L. These concentrations of Se are lower than the maximum allowable concentrations. The concentrations of Se analyzed in BMW samples varied from 70.8 to 217.6 µg/L with an average of 142.5 µg/L. From Fig. 2, the average Se concentration in BMW samples is higher than the maximum allowable concentrations.

Chronic daily intakes (CDI) of potentially toxic elements representing the lifetime average daily dose of exposure to a chemical pollutant were used to evaluate the non-carcinogenic and carcinogenic health risks for adults (as the general population). The CDI (in µg/kg/d) was calculated by the following equation [34]:

$$CDI = \frac{C \times IR \times ET \times EF}{BM \times AT} \quad (1)$$

where C is the concentration of Se analyzed in BMW water samples (µg/L); IR is the ingestion rate of BMW (13 L/y= 0.04 L/d) [35]; ET is the average exposure duration of adults (79 y); EF is the exposure frequency (365 d/y); BM is the average body weight of adults (77 kg) and AT is the average exposure time (79 y x 365 d/y) [36]. The CDI values estimated for adult exposure due to BMW consumption varied from 0.03 to 0.27 µg/kg/d with an average of 0.08 µg/kg/d. These CDI values are lower than the recommended dietary intake of Se in the range of 40 to 400 µg/d (0.57 to 5.71 µg/kg/d).

Table 1. Concentrations of Selenium in drinking water samples

Sample code	The concentration of Se (µg/L)
TW-22	7.6
TW-23	0.8
TW-24	1.8
TW-31	3.9
BMW-1	< 0.5
BMW-2	< 0.5
BMW-3	213.8
BMW-4	< 0.5
BMW-5	117.3
BMW-6	109.4
BMW-7	156.0
BMW-8	< 0.5
BMW-9	< 0.5
BMW-10	< 0.5
BMW-11	141.2
BMW-12	123.0
BMW-13	121.9
BMW-14	160.4
BMW-15	217.6
BMW-16	117.3
BMW-17	< 0.5
BMW-18	161.7
BMW-19	< 0.5
BMW-20	< 0.5
BMW-21	70.8
Average	107.8
Median	119.6
Standard error	16.9
Minimum	0.8
Maximum	217.6
TS	10
TL	10
EC D	10
EPA	50

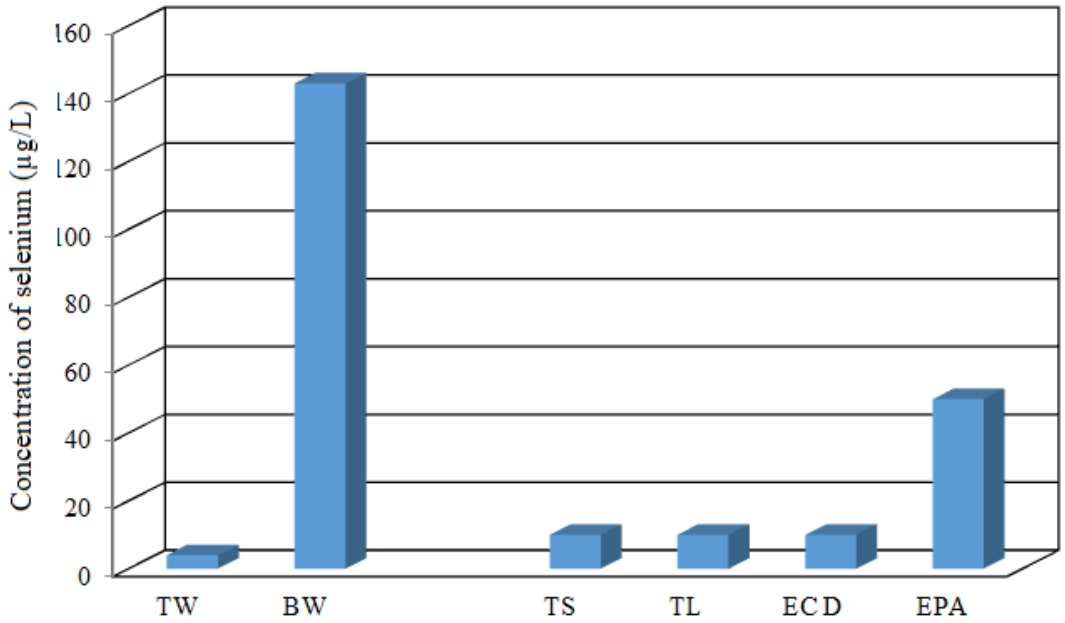


Figure 2. Comparison of the average Se concentration with the maximum allowable concentrations

Conclusion

This study represents the first attempt at the detailed analysis of selenium in the investigated drinking water samples consumed in Kastamonu province. The results showed the Se concentrations analyzed in the bottled plain and fruit-flavored mineral water samples were greater than those analyzed in the tap and bottled spring water samples. The average Se content analyzed in the bottled plain and fruit-flavored mineral water samples is approximately 3–14 times higher than the maximum allowable concentrations. The chronic daily intake results revealed that the investigated bottled mineral water samples could not pose risks to adults who regularly consume these products.

References

[1] Alanazi YJ, Al-Masoud FI, Ababneh ZQ (2021) Assessment of the level of heavy metals in tap water network system of Riyadh, Saudi Arabia. J Environ Sci Public Health 5(1), 137–154.

[2] Al-Taani A, Batayneh A, El-Radaideh N, Al-Momani I, Al-Rawabdeh A (2012). Monitoring of selenium concentrations in major springs of Yarmouk Basin, North Jordan. World Appl Sci J 18(5), 704–714.

[3] Bailey RT (2017) Review: Selenium contamination, fate, and reactive transport in groundwater in relation to human health. Hydrogeol J 25, 1191–1217.

[4] Malhotra M, Pal M, Pal P (2020) A response surface optimized nanofiltration-based system for efficient removal of selenium from drinking water. J Water Process Eng 33, 1–7.

[5] Gebreeyessus GD, Zewge F (2019) A review on environmental selenium issues. SN Appl Sci 1(55), 1–16.

[6] Vinceti M, Filippini T, Cilloni S, Bargellini A, Vergoni AV, Tsatsakis A, Ferrante M (2017) Health risk assessment of environmental selenium: Emerging evidence and challenges (Review). Mol Med Rep 15(5), 3323–3335.

[7] Omanović E, Moderreger H, Kalcher K.(2002) Determination of selenium in drinking water with a simple field device. Anal Lett 35(6), 943–958.

[8] Hudak PF (2009) Elevated fluoride and selenium in West Texas groundwater. Bull Environ Contam Toxicol 82, 39–42.

[9] Bajaj M, Eiche E, Neumann T, Winter J, Gallert C (2011) Hazardous concentrations of selenium in soil and groundwater in North-West India. J Hazard Mater 189(3), 640–646.
[10] Pistón M, Javier S, Zampra RP, Dol I, Moisés K (2011) Automated method for the determination of total arsenic and selenium in natural and drinking water by HG-AAS. Environ Geochem Health 34, 273–278.
[11] Pettine M, McDonald TJ, Sohn M, Anquandah GAK, Zboril R, Sharma VK (2015) A critical review of selenium analysis in natural water samples. Trends Environ Anal Chem 5, 1–7.
[12] Dos Santos E, Junior D, Hermann A, Sturgeon R (2016). Chemical quality of bottled mineral waters from markets of Curitiba-PR-Brazil. Braz Arch Biol Technol 59, 1–24.
[13] Ćurković M, Sipos L, Puntarić D, Dodig-Ćurković K, Pivac N, Kralik K. (2016) Arsenic, copper, molybdenum, and selenium exposure through drinking water in rural Eastern Croatia. Pol J Environ Stud 25(3), 981–992.
[14] Tian Y, Yu C, Zha X, Wu J, Gao X, Feng C, Luo K (2016). Distribution and potential health risks of arsenic, selenium, and fluorine in natural waters in Tibet, China. Water 8(568), 1–16.
[15] Kumkrong P, LeBlanc KL, Mercier PHJ, Mester Z (2018) Selenium analysis in waters. Part 1: Regulations and standard methods. Sci Total Envirot 640(641), 1611–1634.
[16] Golubkina N, Erdenetsogt E, Tarmaeva I, Brown O, Tsegmed S (2018) Selenium and drinking water quality indicators in Mongolia. Environ Sci Pollut Res 25, 28619–28627.
[17] Santos MD, da Silva Júnior FMR, Zurdo DV, Baisch PRM, Muccillo-Baisch AL, Madrid Y (2019). Selenium and mercury concentration in drinking water and food samples from a coal mining area in Brazil. Environ Sci Pollut Res 26, 15510–15517.
[18] Liu Z, Chen B, Li X, Wang L, Xiao H, Liu D (2019) Toxicity assessment of artificially added zinc, selenium, and strontium in water. Sci Total Environ 670, 433–438.
[19] Burylin MY, Romanovskii KA, Kaigorodova EA (2020) Determination of selenium in drinking water by electrothermal atomic absorption spectrometry after photochemical generation, distillation, and preconcentration of its gaseous compounds in a graphite furnace. J Anal Chem 75(11), 1408–1414.
[20] Minuye A, Zewge F, Chandravanshi BS (2020) Levels of selenium in selected river, well and bottled waters in Ethiopia by microwave plasma-atomic emission spectrometry. Bull Chem Soc Ethiop 34(2), 215–226.
[21] Rusiniak P, Ruszczyńska A, Wątor K, Bulska E, Kmiecik E (2020) Methodological aspects concerning sampling and determination of total selenium and selenium species in geothermal waters. Bull Geogr Phys Geogr Ser 18, 5–16.
[22] Kolmykova LI, Korobova EM, Baranchukov VS, Kurnosova IV, Silenok AV, Makarova EM (2021) Chemical composition of groundwater used for drinking in conditions of natural deficiency of iodine and selenium and evaluation of its health effect: the case of Bryansk region (Russia). Environ Geochem Health 43, 4987–5009.
[23] Abejón, RA (2022) Bibliometric analysis of research on selenium in drinking water during the 1990–2021 period: Treatment options for selenium removal. Int J Environ Res Public Health 19(5834), 1–38.
[24] Yanardağ R, Orak H (2000) Total selenium concentration in various waters of Turkey. Environ Technol 22(2), 237–246.
[25] Yabanlı M (2012) Evaluation of trace metals found in bottled waters sold in Izmir Turkey. Ekoloji 21, 84–88.
[26] Tuzen M, Pekiner OZ (2015) Ultrasound-assisted ionic liquid dispersive liquid-liquid microextraction combined with graphite furnace atomic absorption spectrometric for selenium speciation in foods and beverages. Food Chem. 188, 619–624.

[27] Acikkapi AN, Tuzen M, Hazer B (2019) A newly synthesized graft copolymer for magnetic solid phase microextraction of total selenium and its electrothermal atomic absorption spectrometric determination in food and water samples. Food Chem. 284, 1–7.
[28] Atasoy M, Yildiz D, Kula İ (2022) Determination and speciation of selenium in water samples collected from Muğla (Turkey) province by hydride generation atomic absorption spectrometry. J Indian Chem Soc 99, 1–5.
[29] Alzaridi FMNS, Kurnaz A (2020) Determination of concentration levels of toxic elements and radon in drinking waters consumed in Kastamonu province, Turkey. Int J. Environ Anal Chem DOI: 10.1080/03067319.2020.1849664.
[30] Turkish Standard (TS) 266, 2005. Water intended for human consumption. Turkish Standards Institute, Ankara. (in Turkish)
[31] Turkish Legislation. Official Journal, 2013. Regulation on water intended for human consumption, 28580, 07 March 2013, Ankara. (in Turkish)
[32] European Communities Directive, 1998. EU Council directive 98/83/EC of 15 November 1998 on the quality of water intended for human consumption. Off. J. Eur. Communities L 330/32, 05.12.1998.
[33] EPA, 2012. United States Environmental Protection Agency. 2012 Edition of the Drinking Water Standards and Health Advisories, EPA 822-S-12-001, Office of Water U.S. Environmental Protection Agency, Washington, DC, USA.
[34] Dessie BK, Gari SR, Mihret A, Desta AF, Mehari B (2021) Determination and health risk assessment of trace elements in the tap water of two Sub-Cities of Addis Ababa, Ethiopia. Heliyon 7(5), 1–10.
[35] Anonymous (2022) Web page: http://www.drinktech.net/roportaj/beypazari-maden-suyu-yonetim-kurulu-baskani-niyazi-ercan-_117.html.
[36] TÜİK (2022) Web page: https://data.tuik.gov.tr/Bulten/Index?p=Hayat-Tablolari-2017-2019-33711.

MALZEME VE ÇEVRE BİLİMLERİNDE LAZER KIRINIMLI PARÇACIK BOYUT DAĞILIMI ANALİZİ

LASER DIFFRACTION PARTICLE SIZE DISTRIBUTION ANALYSIS IN MATERIALS AND ENVIRONMENTAL SCIENCES

Dr. Ayşe Nur PEKTAŞ
ORCID: 0000-0001-5621-2844
Sivas Cumhuriyet Üniversitesi İleri Teknoloji Uygulama ve Araştırma Merkezi (CUTAM)

ÖZET

Lazer kırınımı, bir lazer ışını dağılmış bir parçacık numunesinden geçerken saçılan ışığın yoğunluğundaki açısal değişimi ölçerek parçacık boyutu dağılımlarını ölçer. Büyük parçacıklar ışığı lazer ışınına göre küçük açılarda saçarken, küçük parçacıklar ışığı geniş açılarda dağıtır. Lazer kırınımı, hacim eşdeğeri bir küre modeli varsayarak, parçacık boyutu dağılımını hesaplamak için Mie ışık saçılımı teorisini kullanır. Açısal saçılma yoğunluğu verileri ile "Mie Işık Saçılımı Teorisi" kullanılarak saçılma modelini oluşturmaktan sorumlu parçacıkların boyutunu hesaplamak için analiz edilir. Böylelikle parçacık boyutu, küre çapına eşdeğer bir hacim olarak hesaplanır.

Mie teorisi, ölçülmekte olan numunenin optik özelliklerinin (kırılma indisi ve hayali bileşen) yanı sıra dağıtıcının(dispersant) kırılma indisi hakkında bilgi gerektirir. Genellikle, dispersant optik özelliklerini yayınlanmış verilerden bulmak nispeten kolaydır ve birçok modern araç, ortak dispersant içeren yerleşik veri tabanlarına sahiptir. Dolayısıyla Mie modeli ile hesaplama yapabilmek için hem malzemenin hem de dispersantın kırılma indisinin bilinmesi gereklidir. Optik özelliklerin bilinmediği numuneler için, ölçebilir veya modellenen veriler ile numune için toplanan gerçek veriler arasındaki uyuma dayanan yinelemeli bir yaklaşım kullanarak tahmin edebilir. Basitleştirilmiş bu yaklaşım, malzemenin optik özellikleri hakkında bilgi gerektirmeyen Fraunhofer yaklaşımıdır. Bu yaklaşım ile büyük parçacıklar için doğru sonuçlar rahatlıkla elde edilebilir. Bununla birlikte, partikülleri 50µm'nin altında olabilecek numunelerle çalışırken veya partiküllerin nispeten şeffaf olduğu durumlarda ise çok doğru sonuçlar vermeyebilir.

Parçacık boyut analizinde, malzemenin mukavemeti, kimyasal reaktifliği, opaklığı, akışkanlığı gibi özellikleri malzemenin içindeki tanecik boyutu karakteristiklerine bağlıdır. Parçacık boyut analizi seramikler, toz metalürjisi, mineral prosesleri gibi malzeme bilimlerinde, toprak tane boyut analizi gibi jeoloji analizlerinde ve farmasötik kimya alanı gibi çok geniş yelpazede kullanılabilen bir analiz türüdür.

Anahtar Kelimeler: Lazer kırınımı, Mie, parçacık boyutu

ABSTRACT

Laser diffraction measures particle size distributions by measuring the angular change in the intensity of the scattered light as a laser beam passes through a sample of scattered particles. Large particles scatter light at small angles relative to the laser beam, while small particles scatter light at wide angles. Laser diffraction uses Mie light scattering theory to calculate the particle size distribution, assuming a volume-equivalent sphere model. The angular scattering intensity data is analyzed using "Mie Light Scattering Theory" to calculate the size of the particles responsible for forming the scattering pattern. Thus, the particle size is calculated as a volume equivalent to the sphere diameter.

Mie theory requires information about the optical properties (refractive index and imaginary component) of the sample being measured, as well as the refractive index of the dispersant.

Generally, dispersant optical properties are relatively easy to find from published data, and many modern tools have built-in databases of common dispersants. Therefore, in order to be able to calculate with the Mie model, it is necessary to know the refractive index of both the material and the dispersant. For samples where the optical properties are unknown, it can measure or estimate using an iterative approach based on the fit between the modeled data and the actual data collected for the sample. This simplified approach is the Fraunhofer approach, which does not require knowledge of the optical properties of the material. With this approach, accurate results for large particles can be easily obtained. However, it may not give very accurate results when working with samples that may have particles below 50µm or where the particles are relatively transparent.

In particle size analysis, properties such as strength, chemical reactivity, opacity, and fluidity of the material depend on the particle size characteristics in the material. Particle size analysis is a type of analysis that can be used in a wide range of materials sciences such as ceramics, powder metallurgy, mineral processes, geology analysis such as soil particle size analysis, and pharmaceutical chemistry.

Keywords: Laser diffraction, Mie, particle size

1. GİRİŞ

En temel düzeyde, bir parçacığı, bir maddenin ayrık bir alt bölümü olarak tanımlayabiliriz. Bu tanımı, fiziksel boyutları nanometre altı ile birkaç milimetre arasında değişen katı partikülleri, sıvı damlacıkları veya gaz kabarcıklarını içerecek şekilde ele alabiliriz. Parçacıklardan oluşan en yaygın malzeme türleri şunlardır:

- tozlar ve granüller örn. pigmentler, çimento, farmasötik bileşenler
- süspansiyonlar, emülsiyonlar ve bulamaçlar örn. aşılar, süt, maden çamuru
- aerosoller ve spreyler örn. astım inhalerleri, bitki koruma spreyleri

Birçok endüstrinin rutin olarak işlerinde parçacık karakterizasyonu kullanmasının iki ana nedeni vardır,

1. Ürün kalitesinin daha iyi kontrolü,
2. Ürünlerin, bileşenlerin ve süreçlerin daha iyi anlaşılması.

Ürün kalitesini kontrol etmenin yanı sıra, partikül özelliklerinin ürünlerinizi, içeriklerinizi ve süreçlerinizi nasıl etkilediğini daha iyi anlamak, şunları yapmanızı sağlar:

- ürün performansını iyileştirmek,
- üretim ve tedarik sorunlarını gidermek,
- üretim süreçlerinin verimliliğini optimize etmek,
- çıktıyı artırmak veya verimi artırmak,
- rekabette bir adım önde olmak

1.1. Hangi parçacık özelliklerini ölçmek önemlidir?

Kimyasal bileşime ek olarak, partiküllü malzemelerin davranışına genellikle bileşen partiküllerin fiziksel özellikleri hakimdir. Bunlar, örneğin reaksiyon ve çözünme hızları, bileşenlerin ne kadar kolay aktığı ve karıştığı veya sıkıştırılabilirlik ve aşındırıcılık gibi çok çeşitli malzeme özelliklerini etkileyebilir. Üretim ve geliştirme açısından, ölçülmesi gereken en önemli fiziksel özelliklerden bazıları şunlardır:

- parçacık boyutu
- parçacık şekli
- yüzey özellikleri
- Mekanik özellikler
- yük özellikleri
- mikro yapı

İlgilenilen malzemeye bağlı olarak, bunların bazıları veya tümü önemli olabilir ve hatta birbirleriyle ilişkili olabilir: ör. yüzey alanı ve parçacık boyutu.

Partikül boyutu ve şekli, ara ve nihai ürünlerin kalitesini doğrudan etkilediği için partikül oluşumu ve işlenmesi sırasında önemli bir rol oynar. Bu nedenle, modern toz endüstrilerinde tercihen hat içi veya çevrim içi enstrümanlar kullanılarak bu işlemlerin uygun şekilde izlenmesi ve kontrolüne çok ihtiyaç duyulmaktadır[1].

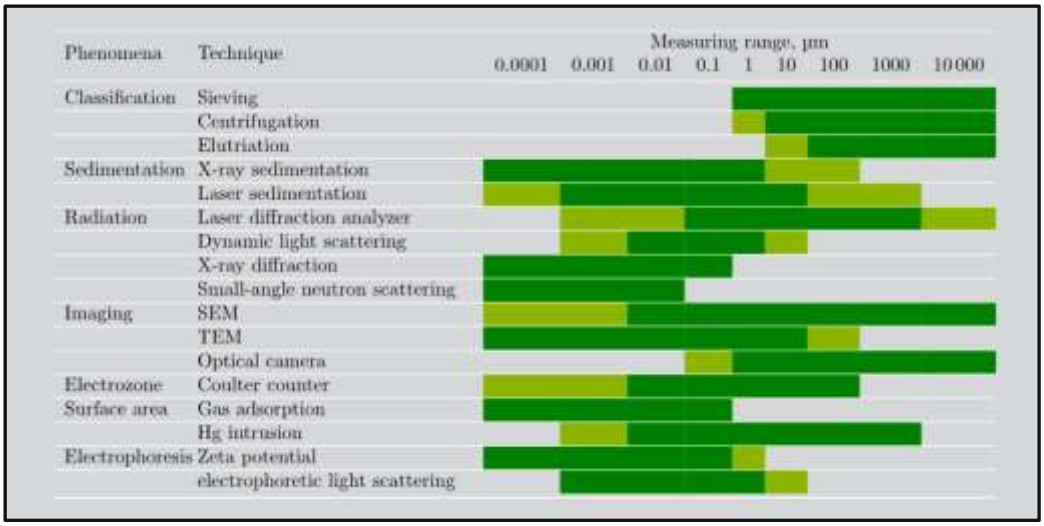
1.2. Lazer Kırınımı İle Parçacık Boyut Dağılımı Ölçme

Lazer kırınım ölçümlerinde, uygulanabilir saçılma modellerine dayalı olarak saçılma açısının ve ışığın dalga boyunun bir fonksiyonu olarak saçılma yoğunluğunun ölçümleri yoluyla parçacık boyutu dağılımı hakkında bilgi elde edilir. Bu, deneysel düzenek veya cihaz doğru bir şekilde kurulduğunda, kalibrasyon veya ölçeklendirmenin gerekli olmadığı mutlak bir yöntemdir. Son yirmi yılda, lazer kırınımı endüstriyel partikülleri boyutlandırmak için popüler ve önemli bir fiziksel araç haline gelmiştir. Lazer kırınımı, birkaç milimetreden daha küçük parçacıkların boyutlandırılmasında eleme ve çökeltme gibi geleneksel yöntemlerin büyük ölçüde yerini almış ve onlarca nanometreden daha büyük parçacıklar için optik ve elektron mikroskobunun yerini almıştır. Esas olarak, kullanım kolaylığı ve hızlı çalışması, yüksek tekrarlanabilirliği, ve nanometreden milimetreye kadar neredeyse beş büyüklük sırasını kapsayan son derece geniş bir dinamik boyut aralığı ile teknolojinin avantajlarını sunmaktadır[2].

Parçacık boyutu analizi için, parçacıklar tarafından ileri yönde saçılan lazer ışığının ölçümüne dayanan optik aletler artık laboratuvarda popüler ve standart bir teknik haline gelmiştir. İleri yönde saçılan ışık genellikle kırınım teorisi ile doğru bir şekilde tahmin edilebildiğinden, alet sınıfı genellikle lazer kırınım aletleri olarak bilinir hale gelmiştir. Diğer partikül boyutlandırma teknikleri ile karşılaştırıldığında, lazer kırınımı yüksek hız, iyi güvenilirlik ve yüksek tekrarlanabilirlik avantajına sahiptir. Hem katı-sıvı süspansiyonlarda hem de gaz-katı sistemlerde, doğrudan hat içi proses ve birçok toz prosesinin kalite kontrolü için giderek daha fazla uygulanmaktadır[3]. Bununla birlikte, dedektörlerden gelen zaman ortalamalı sinyalleri kullanan lazer kırınımındaki mevcut uygulama, ana parçacık boyutu dağılımı PSD'nin dışında kalan küçük miktarlardaki büyük parçacıklara duyarlı değildir. Bu, bu büyük parçacıkların olduğu uygulamalarda kullanımını sınırlar. küçük konsantrasyonlarda ürün kalitesinin bozulmasına neden olur. Bir kaplama uygulamasında kullanılacak olan hemen hemen tüm ürünler bu tür kontaminasyona karşı hassastır [3]

Lazer kırınımı ile partikül boyutlandırma da partikül şeklinden etkilenir. Normalde, kullanılan optik model, parçacıkların küresel olduğunu varsayar. Bununla birlikte, küresel olmayan parçacıklar, görünen PSD'de sistematik bir hatayla sonuçlanabilecek sapmalı saçılma modelleri gösterir[4,5]. Örneğin, gerçekte var olmasalar da kaba parçacıkların varlığı belirtilebilir. Alternatif olarak, geniş açılarla saçılan ışık bazen gerçekte var olmayan küçük parçacıkların varlığını gösterir. Bu nedenle, eğer biliniyorsa partikül şekli, doğru PSD'yi elde etmek için tercihen optik modele dahil edilmelidir. Ayrıca, partikül şeklinin kendisi, partiküllü maddenin karakterizasyonunda artan bir ilgidir. Lazer kırınım ölçümlerinde, partikül konsantrasyonu bilgisi, partikül şekli hakkında bazı bilgiler sağlayabilir. Örneğin, Brown ve Felton, farklı şekiller için “Fraunhofer modeli” aracılığıyla hesaplanan partikül konsantrasyonlarını gerçek, bilinen konsantrasyonla karşılaştırarak partikül şekli hakkında sonuçlar çıkarmıştır.

Parçacık boyutu analizörleri, temel fiziko-kimyasal özelliklerle ilgili altı kategoriye ayrılır: sınıflandırma, tortulaşma, radyasyon, görüntüleme, elektro-zone algılama ve zeta potansiyeli (Şekil 1)[6]. Görüntüleme, en geniş parçacık boyutu aralığını kapsar ve subnanometrik ölçeğe (0,1 nm) ulaşır. Çoğu teknik, bir veya daha fazla teknolojiyi birleştirerek aralıklarını genişletir. Standart aralık, Şekil 1'deki koyu yeşil alanlara karşılık gelirken, genişletilmiş aralık daha açık bir yeşil ile renklendirilmiştir.



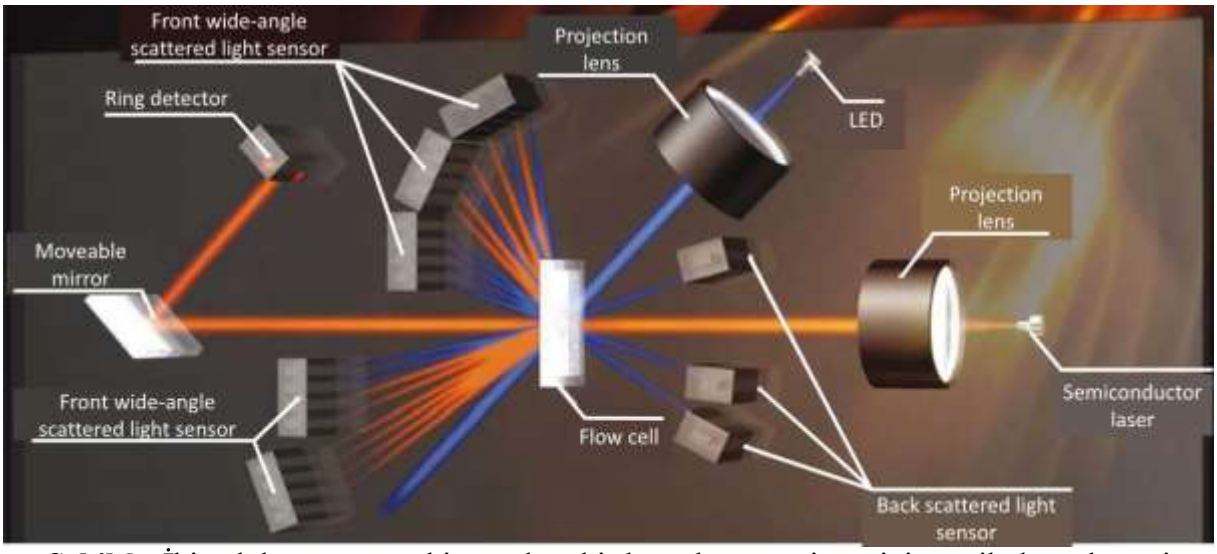
Şekil 1. Partikül boyutu ölçüm yöntemleri ve aralıkları (Li ve ark., 2019)

Bazı teknikler nanometrik boyutu ölçer ancak henüz parçacık boyutu dağılımını ölçmeye yönelik değildir[7]. Yerçekimi sedimantasyon, merkezkaç sedimantasyon ve hat başlangıçlı santrifüj sedimantasyon, kaba parçacıklar için en uygun olanıdır ancak diğer bazı tekniklere göre zaman alıcı ve pahalıdır. Santrifüjleme, bir hava yıkama şeklidir ve partikül boyutunu 2µm'ye kadar ölçerken, dp> 30µm için sıvı fazda yıkama madencilik endüstrisinde yaygın olarak uygulanır. Bir fotosedimentometre, çökelme işlemi sırasında parçacıkların Brownian hareketini izler ve 0,01–1000µm için geçerlidir. Bir x-ışını fotosedimentometresi, ışık yerine x-ışınları uygular, bu da hassasiyetini artırır. Bunun yanı sıra, 3D görüntüleme için röntgen tomografisi kullanılmıştır ve partikül şeklini de belirler, ancak teknik zaman alıcıdır.

Gelen ışığın dalga boyu, lazer kırınım analizörlerinin (LDA) alt sınırını 0,01-0,1 µm ile sınırlıdır, ancak çoklu ışık kaynakları ve gelişmiş algoritmik yazılım ile alt sınır 0,001µm'ye ulaşabilir. XRD ve küçük açılı nötron saçılması, kristal yapıyı tespit ederken aynı zamanda kristalit boyutunu da tahmin eder. Cihazın numuneleri analiz etmesi saniyeler alır ve tekrarlanabilirlik iyidir. Elektro-zone algılama yöntemleri (örneğin Coulter Counter) LDA kadar hızlıdır ancak bir açıklıktan akan parçacıkları askıya almaları gerektiğinden daha dar bir aralığa uygulanır. Porozimetreler, zeta potansiyel analizörleri gibi elektroforetik aletler gibi partikül boyutlarını da tahmin eder. Bu aletlerin menzili, LDA ve görüntüleme tekniklerinden çok daha küçüktür. Porozimetrinin sınırlamalarından biri, numunelerin çalıştırılmasının saatler sürmesidir.

Parçacık şekli, aletlerin dp'yi nasıl belirlediğini de etkiler: görüntüleme teknikleri, yansıtılan alanı ölçer; elektron algılama hacme göre atanır ve sedimantasyon teknikleri bunu partikül terminal hızına göre atar[8]. Geniş bir parçacık boyutu aralığı, LDA ve damlacıkları ölçmenin[9] yanı sıra, analizör saniyeler içinde bir dağılım oluşturduğundan, operatörler günde yüzlerce testi tamamlayabilir. Tüm LDA'lar uluslararası standartları takip eder ve kabul edilebilir kesinlik ve tekrarlanabilirliğe sahiptir. LDA teknolojisi 1970'lerde geliştirildi ve o zamandan beri birçok firma tarafından üretilmiştir[10].

Cihaz, ışık kaynakları, bir örnek sunum sistemi, bir dizi dedektör ve yazılım ve algoritmalardan oluşur[7].



Şekil 2. İki ışık kaynağına sahip modern bir lazer kırınım sisteminin optik düzenlemesi (Li ve ark., 2019)

Işık kaynağı

Lazerler ve ışık yayan diyotlar (LED) parlak, tek renkli ışık kaynaklarının yerini almıştır. Lazerlerin ve LED'lerin dayanıklılığı ve ömrü akkor lambalardan çok daha iyidir. Yeni cihazlar ışını daha kısa yol uzunluklarında örneklere yönlendirir, böylece daha kompakt olurlar. Menzili artırmak için cihazlar birden fazla ışık kaynağına sahiptir: daha kısa dalga boylu kaynaklar daha küçük parçacıklar için en iyisidir ve daha uzun dalga boyları daha büyük parçacıklar için en iyisidir.

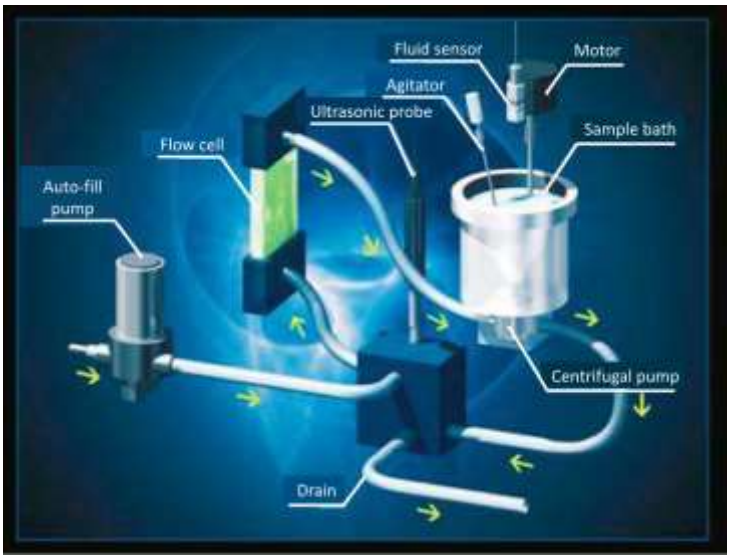
Şekil 2'de sağdaki yarı iletken lazer, merkezdeki akış merkezinde numuneyi aydınlatır. Numunenin etrafındaki bir dizi ayrı geniş açılı dedektörden oluşan bir halka, bir aynadan sola yansıyan saçılan ışığı ölçer. İkinci ışık kaynağı sağ üsttedir ve aynı geniş açılı dedektör setinden yararlanır.

Numunenin sisteme verilmesi

Numune, toz ya bir sıvı içinde dağıtılır ve dedektör dizisine pompalanır ya da bir hava akımıyla dedektöre taşınır. Kuru analiz için, bir vakum pompası parçacıkları numune hücresi boyunca sürükler ve hava, aglomeraları ayırır ve analizden önce dağılır. Islak analiz, sıvının tozu dağıtması için uygun bir çözücü seçerek, bir yüzey aktif madde ekleyerek veya sonikasyon yaparak aglomeraların oluşmasını önlemesini gerektirir (numune banyosu gibi). Elektro-zone aletlerinde olduğu gibi, numune banyosu partikülleri askıya almalı ve bunların çökmesini veya yüzmesini önlemelidir. Bir sıvı pompası, akış hücresini sabit bir hızda besler ama aynı zamanda parçacıkları asılı tutmak için numune banyosunu da karıştırabilir. Alternatif olarak, bağımsız bir yıldız çubuğu, seyreltik süspansiyonu çalkalar; ancak saniyeler içinde çökmesi muhtemel daha büyük partiküller için bağımsız bir pompa, partiküllerin asılı kalmasını sağlamak için sıvıyı dolaştırır (Şekil 3).

Dedektörler

LDA için en uygun olan silikon detektörlerdir. Ya daha geniş açılarda yeterli alan olduğunda ayrı dedektörler olarak dağıtılır ya da dar saçılma açıları için bir dizi eşmerkezli halkaya monte edilir. Parçacıklar hücre içinde yuvarlandığından, saçılmayı azimut açısının bir fonksiyonu olarak tespit etmenin pratik bir faydası yoktur.



Şekil 3. Modern lazer kırınım akış sistemi (Li ve ark., 2019)

Soldaki bir otomatik doldurma pompası sıvıyı numune banyosuna verir ve ardından sıvının yüksekliği ayar noktasına ulaştığında kapanır. Operatörler tozu banyoya ekler ve bir karıştırma çubuğu seyreltik bulamacı karıştırır. Bir santrifüj pompa, malzemeyi numune hücresi boyunca hareket ettirir ve bir ultrasonik sonda, hücrenin yukarısındaki gevşek aglomeraları parçalar. Testin sonunda, otomatik doldurma pompası sistemi durular.

1.3. Lazer Kırınım Analizinin Arka Plan Teorisi

LDA'nın arkasındaki bilimsel teori, farklı açılarda ölçülen bir parçacığın saçılan yoğunluğunu parçacık boyutuna (veya dağılımına) bağlayan "**Fraunhofer kırınımı"** veya "**Mie saçılması"** teorisine dayanır. Fraunhofer kırınım teorisi, aslında, Mie saçılma teorisinin bir yaklaşımıdır. Bununla birlikte, yalnızca gelen dalga boyunun en az 10 katı olan parçacıklar için ve saçılma açısı 30°'dan küçük olduğunda geçerlidir. Fraunhofer kırınım teorisi, 20µm'den daha ince parçacıklar için kabul edilemez hatalara neden olur. Mie teorisi, daha küçük parçacıkların daha geniş bir ışık yoğunluğu dağılımı aralığı için geçerlidir; ancak, kırılma indeksleri gereklidir. Herhangi bir çaptaki küresel, homojen, izotropik ve manyetik olmayan parçacıkların, emici olmayan bir ortamdan saçılma yoğunluğu için kesin çözümdür. Yönlendirilmiş bir ışık demeti tarafından aydınlatılan küresel bir parçacıktan saçılma modelinin matematiksel formülasyonu şöyledir:

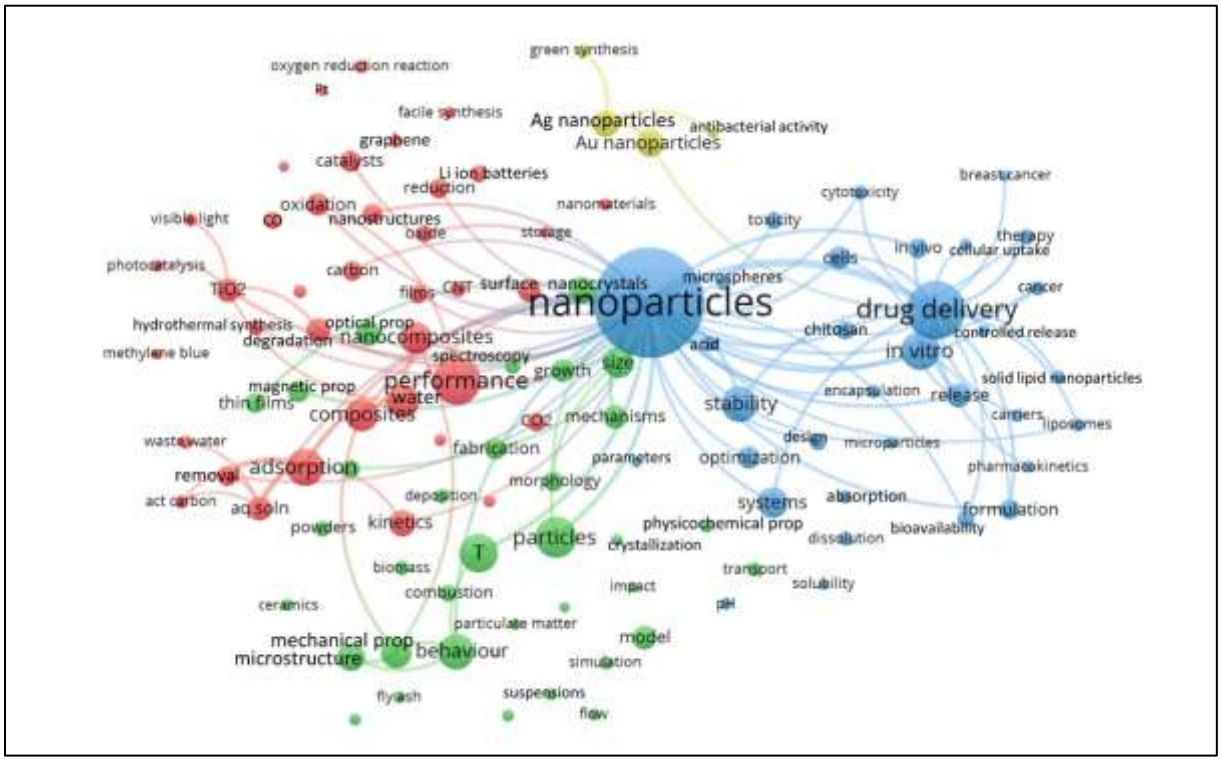
$$I(\theta, \varepsilon, m) = \frac{\lambda^2}{8\pi^2 r^2} I_0 \int_{\theta_1}^{\theta_2} [|S_1(\theta, \varepsilon, m)|^2 + |S_2(\theta, \varepsilon, m)|^2] \mathrm{d}\theta$$

Burada ***I*** ışık yoğunluğu, ***ε*** bir parçacık boyutu faktörüdür **(ε= πD/ λ)**, (λ gelen ışığın dalga boyudur), **θ** kırınım açısıdır, **m** parçacıkların karmaşık kırılma indisidir (m=n+*ik*), **r** parçacık ve dedektör arasındaki mesafe ve **S1** ve **S2**, **Bessel ve Legendre Fonksiyonları Denklemi'**nden oluşur. Parçacıkların karmaşık kırılma indisi, saçılma kesitini veya modelini etkiler ve böylece LDA alt algılama sınırını etkiler.

2. UYGULAMALAR

Partikül boyutu ve LDA, WOS'da en çok alıntı yapılan deneysel yöntemler arasındadır. Şekil 4'te görüleceği üzere en büyük küme (mavi), nanoparçacıkları ve sağlık ve farmakolojiyle ilgili diğer konuları içerir: tıbbi nebulizasyon, kistik fibrozis, tobramisin, akciğerler ve ilaç dağıtımı. Sedimantasyon, aglomerasyon ve toprak, mikroskopi ve görüntü analizi, neredeyse

mavi küme kadar araştırmaya sahip olan kırmızı kümeye aittir. En sık kullanılan anahtar kelimeler performans, adsorpsiyon, kompozitler ve sudur. Konuların çoğu aynı zamanda katalizi de içerir. Fizikokimyasal ve mekanik özellikler üçüncü en büyük kümeyi (yeşil) oluşturur. Bu kümedeki araştırmalar, tozlar, partikül stabilitesi ve reoloji, emülsiyonlar, ufalama ve stabilite dahil olmak üzere dispersiyonlarla ilgilidir. Son olarak, en küçük küme (sarı) antibakteriyel özellikler, altın ve gümüş nanoparçacıklar ve yeşil kimya ile ilgilidir.



ŞekilHata! Belgede belirtilen stilde metne rastlanmadı. **4.** Parçacık boyutu analizlerinin bibliyometrik haritası (Li ve ark., 2019)

3. SINIRLAMALAR

LDA, lazer, numune hücresinden geçerken gelen radyasyon saçılma açısını ve yoğunluğunu ölçerek partikül boyutunu bildirir. Büyük parçacıklar ışığı küçük bir açıyla yüksek yoğunlukta saçarken, daha küçük parçacıklar ışığı daha büyük bir açıyla ancak daha az yoğunlukla dağıtır. Bir bilgisayar algoritması, açısal saçılma yoğunluğu verilerini analiz eder. Genel olarak, LDA testi Fraunhofer teorisine[11] veya Mie teorisine dayalı olarak gerçekleştirir[12]. LDA için ISO standardı, 20µm'nin altındaki parçacıklar için Mie teorisini önerir. Bununla birlikte, LDA ortalama partikül boyutunu yaklaşık %10-20'ye kadar olduğundan daha az tahmin edebilir. LDA, boyuta bağlı hacim faktörü ile sınırlıdır ve yüksek konsantrasyon dispersiyonlarının PSD'sini yeniden üretemez.

Hem Mie teorisi hem de Fraunhofer teorisi, tüm parçacıkların küresel olduğunu varsayar. Parçacık şekli sadece ışığın nasıl dağıldığını etkilemekle kalmaz, aynı zamanda şekil ve doku kırılma indisinin (RI) hayali kısmını etkiler. Ayrıca cihazlar, sudan daha düşük RI değerine sahip polistiren partikülleri ile kalibre edilir. Bu nedenle, LDA, katı-sıvı aerosol damlacıkları gibi polistiren partiküllerinden daha düşük RI'ye sahip tozları diikkate almayacaktır. Mie teorisi, birçoğu yayınlanmış literatürde veya cihaz veritabanında bulunan bir RI gerektirir. Karışımlar ve bilinmeyen malzemeler için RI'nin belirlenmesi, LDA'nın bir sınırlamasıdır. Bazı gelişmiş araçlar, kullanıcıların RI'yi gerçek verilerle model verilerine uygunluğu karşılaştıran bir makine öğrenimi yaklaşımına dayalı olarak tahmin etmelerine olanak tanır[13].

4. SONUÇ

Parçacık boyutu en önemli fizikokimyasal özelliklerden biridir. LDA, PSD'yi ölçmek için en etkili analitik teknikler arasındadır, çünkü en geniş aralığa sahiptir ve çalıştırılması (katı örnekleme, hazırlama ve bakımın azaltılması) yalnızca saniyeler alır ve en fazla birkaç gram toz gerektirir. Algılama aralığı 10–7500 nm kadar düşük bir aralıkta değişir.

Mie teorisi, hafif ve opak katılar arasındaki etkileşimi karakterize eden analitik bir çözümdür. Bununla birlikte, kompozit tozlar ve karışımlar için belirsiz olan bir kırılma indisi gerektirir. Ayrıca tüm parçacıkların küresel olduğunu varsayar, bu da hataya neden olur ve parçacık boyutunu olduğundan fazla tahmin etme eğiliminde olur. Gelecekteki cihazlar çoklu dedektörler (yeşil, kırmızı ve mavi ışık) geliştirmeye devam edecek, dinamik ışık saçan enstrüman uygulamaları daha da büyüyecek ve gelecek teknolojiler hem doğruluğu hem de menzili artıracatır[7].

5. REFERANSLAR

1. Harvill TL, Holve DJ. In-Process Particle Size Distribution Measurements and Control. *Particle & Particle Systems Characterization*. 1993;10(5):262-265. doi:https://doi.org/10.1002/ppsc.19930100508
2. Laser Diffraction. In: Xu R, Scarlett B, eds. *Particle Characterization: Light Scattering Methods*. Springer Netherlands; 2002:111-181.
3. Ma Z, Merkus HG, de Smet JGAE, Heffels C, Scarlett B. New developments in particle characterization by laser diffraction: size and shape. *Powder Technology*. 2000/08/21/ 2000;111(1):66-78. doi:https://doi.org/10.1016/S0032-5910(00)00242-4
4. Mühlenweg H, Hirleman ED. Laser Diffraction Spectroscopy: Influence of Particle Shape and a Shape Adaptation Technique. *Particle & Particle Systems Characterization*. 1998;15(4):163-169. doi:https://doi.org/10.1002/(SICI)1521-4117(199808)15:4<163::AID-PPSC163>3.0.CO;2-8
5. Heffels CMG, Verheijen PJT, Heitzmann D, Scarlett B. Correction of the Effect of Particle Shape on the size distribution measured with a laser diffraction instrument. *Particle & Particle Systems Characterization*. 1996;13(5):271-279. doi:https://doi.org/10.1002/ppsc.19960130504
6. Smith KA, Mullins CE. *Soil and environmental analysis*. Marcel Dekker Incorporated; 2000.
7. Li H, Li J, Bodycomb J, Patience GS. Experimental Methods in Chemical Engineering: Particle Size Distribution by Laser Diffraction—PSD. *The Canadian Journal of Chemical Engineering*. 2019;97(7):1974-1981. doi:https://doi.org/10.1002/cjce.23480
8. Patience GS. *Experimental methods and instrumentation for chemical engineers*. Elsevier; 2017.
9. Wan B, Fradette L. Phase inversion of a solid-stabilized emulsion: Effect of particle concentration. *The Canadian Journal of Chemical Engineering*. 2017;95(10):1925-1933. doi:https://doi.org/10.1002/cjce.22892
10. Weiss EL, Frock HN. Rapid analysis of particle size distributions by laser light scattering. *Powder Technology*. 1976;14(2):287-293.
11. Pye K, Blott SJ. Particle size analysis of sediments, soils and related particulate materials for forensic purposes using laser granulometry. *Forensic Science International*. 2004;144(1):19-27.
12. Buurman P, Pape T, Reijneveld J, De Jong F, Van Gelder E. Laser-diffraction and pipette-method grain sizing of Dutch sediments: correlations for fine fractions of marine, fluvial, and loess samples. *Netherlands Journal of Geosciences*. 2001;80(2):49-57.
13. Panerati J, Schnellmann MA, Patience C, Beltrame G, Patience GS. Experimental methods in chemical engineering: Artificial neural networks–ANNs. *The Canadian Journal of Chemical Engineering*. 2019;97(9):2372-2382.

KURAKLIK STRESİ ALTINDAKİ MISIR FİDELERİNE ASETON O-(2-NAFTİLSÜLFONİL)OKSİM ÖN UYGULAMASININ BİYOKİMYASAL PARAMETRELER ÜZERİNDEKİ ETKİLERİNİN İNCELENMESİ

INVESTIGATION OF THE EFFECTS OF ACETONE O-(2-NAPHTHYLSULFONYL)OXIME PRETREATMENT ON BIOCHEMICAL PARAMETERS OF MAİZE SEEDLINGS UNDER DROUGHT STRESS

Dr. Öğr. Üyesi, Fuat YETİŞSİN
Orcid no: 0000-0001-6085-7610
Muş Alparslan University, Technical Sciences Vocational School

ÖZET

Oksimlerin genel ve özel metabolizmanın işleyişinde önemli fonksiyonlara sahip olduğu bilinmektedir. Sentetik bir sülfonat türevi olan aseton o-(2-naftilsülfonil)oksim (ANSO)'in ön uygulamasının mısır fidelerinin kuraklık stresi altında bazı fizyolojik ve biyokimyasal parametreleri üzerine etkilerini belirlemek mevcut çalışmanın temel amacıdır. Deney düzeneği: 18 saat distile su Kontrol (C), 6 saat 0,3 mM ANSO+12 saat distile su (ANSO), 6 saat distile su+12 saat %3 PEG (D) ve 6 saat ANSO+12 saat %3 PEG (ANSO+D). ANSO uygulamasında kontrole göre H_2O_2 içeriği azalırken, ANSO+D uygulamasında D'ye göre H_2O_2 içeriğinde önemli bir azalış belirlendi. Tek başına ANSO ile kontrol uygulamaları arasında MDA içeriği açısından önemli bir fark belirlenemezken, ANSO+D uygulamasında D'ye göre MDA içeriğinin önemli oranda azaldığı görüldü. Bu durumun temelinde yatan temel faktör olan Antioksidan sistem enzimlerinden süper oksit dismutaz (SOD), katalaz (CAT), Askorbat peroksidaz (APX) ve guaikol peroksidaz (GPX) enzimlerinin aktivitelerinde yeniden yapılan düzenlemelere göre; ANSO uygulamasında kontrole göre SOD aktivitesi azalırken, ANSO+D uygulamasında D'ye göre SOD aktivitesinde önemli bir azalış gözlendi. GPX aktivitesi açısından ANSO uygulamasında kontrole göre GPX aktivitesi azalırken, ANSO+D uygulamasında D'ye göre GPX aktivitesinde önemli bir artış kaydedildi. APX ve CAT enzim aktiviteleri benzer tepkilerle, ANSO uygulamasında kontrole göre bir fark gözlenmezken, ANSO+D uygulamasında D'ye göre APX ve CAT aktivitelerinde önemli bir artış kaydedildi. Böylece antioksidan sistemin ROS'ların süpürülmesine aktif olarak katıldığı belirlendi. Sonuç olarak, ANSO'nun kuraklık stresi altındaki mısır fidelerine ön uygulanmasının radikal oluşumunu engellemesi ile membran hasarının hafifletilmesinin ANSO'nun antioksidan özelliklerinden kaynaklandığını düşündürmektedir.

Anahtar Kelimeler: Aseton o-(2-naftilsülfonil)oksim, Antioksidan Sistem, Kuraklık Stresi, Mısır fideleri.

ABSTRACT

It is known that oximes have important functions in the functioning of general and special metabolism. The main aim of the current study is to determine the effects of pre-application of acetone o-(2-naphthylsulfonyl)oxime (ANSO), a synthetic sulfonate derivative, on some physiological and biochemical parameters of maize seedlings under drought stress. Experimental setup: 18 hours distilled water Control (C), 6 hours 0.3 mM ANSO+12 hours distilled water (ANSO), 6 hours distilled water+12 hours 3% PEG (D), and 6 hours ANSO+12 hours 3% PEG (ANSO+D). While H_2O_2 content decreased in ANSO application compared to control, a significant decrease in H_2O_2 content was determined in ANSO+D application compared to D. While no significant difference could be determined between ANSO alone and control applications in terms of MDA content, it was observed that MDA

content decreased significantly in ANSO+D application compared to D. According to the rearrangements in the activities of the antioxidant system enzymes, superoxide dismutase (SOD), catalase (CAT), Ascorbate peroxidase (APX) and guaicol peroxidase (GPX), which is the main factor underlying this situation; While SOD activity decreased in ANSO application compared to control, a significant decrease in SOD activity was observed in ANSO+D application compared to D. In terms of GPX activity, GPX activity decreased in ANSO application compared to control, while a significant increase in GPX activity compared to D was recorded in ANSO+D application. APX and CAT enzyme activities with similar responses, while no difference was observed in ANSO application compared to control, a significant increase was observed in APX and CAT activities compared to D in ANSO+D application. Thus, it was determined that the antioxidant system actively participates in the scavenging of ROS. In conclusion, it is suggested that the prevention of radical formation and mitigation of membrane damage by pre-application of ANSO to corn seedlings under drought stress is due to the antioxidant properties of ANSO.

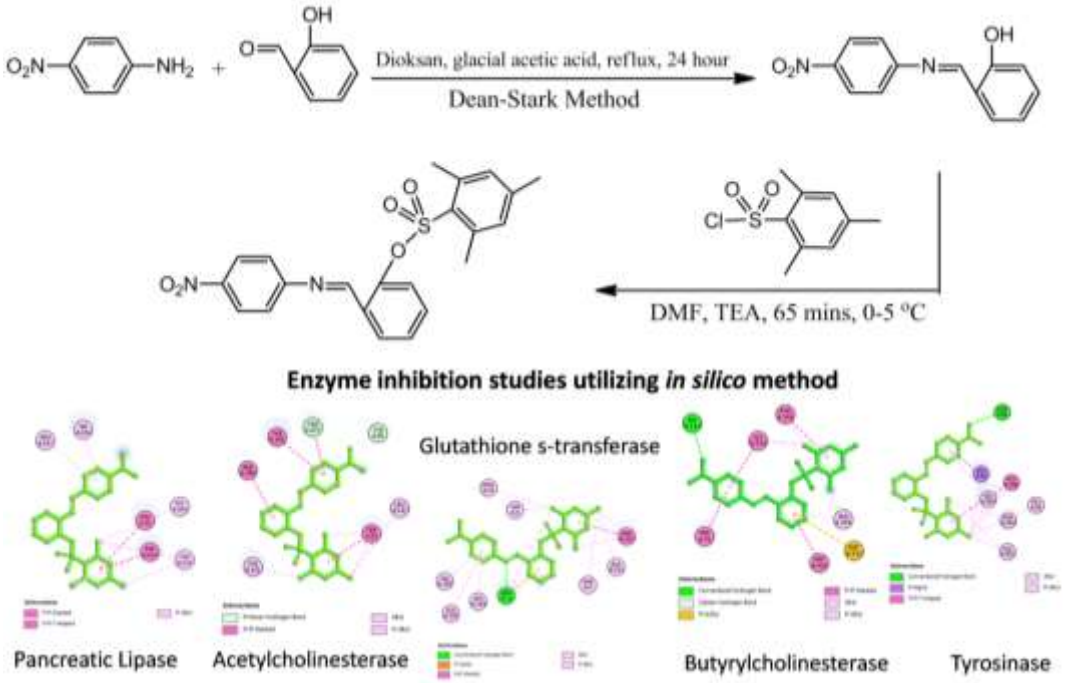
Keywords: Acetone o-(2-naphthylsulfonyl)oxime, Antioxidant System, Drought Stress, Maize seedlings.

AZOMETİN İÇEREN YENİ ARİL NİTRO-SÜLFONAT HİBRİT BİLEŞİĞİNİN SENTEZİ, KARAKTERİZASYONU VE MOLEKÜLER YERLEŞTİRME ÇALIŞMALARI

Dr. Adem KORKMAZ
ORCID:0000-0002-0345-5794
Muş Alparslan Üniversitesi, Sağlık Bilimleri Fakültesi

ÖZET

Dean-Stark yöntemiyle hazırlanan 2-(((4-nitrophenyl)imino)methyl)phenol bileşiği ve 2,4,6-trimethylbenzenesulfonyl chloride bileşiğinden ılıman reaksiyon koşulları altında yeni 2-(((4-nitrophenyl)imino)methyl)phenyl 2,4,6-trimethylbenzenesulfonate bileşiği sentezlenmiştir (Şekil 1). 2-(((4-nitrofenil)imino)metil)fenil 2,4,6-trimetilbenzensülfonat bileşiği ^{1}H NMR ve ^{13}C NMR ile karakterize edilmiştir. Daha sonra bu bileşiğin in silico yöntem kullanılarak asetilkolinesteraz, butirilkolinesterez, glutatyon s-trensferaz, trosinaz, ve pankreatik lipaz enzimleriyle enzim inhibisyonu çalışılmıştır. Yeni 2-(((4-nitrophenyl)imino)methyl)phenyl 2,4,6-trimethylbenzenesulfonate bileşiğinin asetilkolinesteraz, butirilkolinesterez, glutatyon s-trensferaz, trosinaz, ve pankreatik lipaz enzimleriyle moleküler doking skorları sırasıyla -11.0, -5.2, -6.7, -7.8 ve -9.8 kcal/mol olarak bulunmuştur.



Şekil 1. Azometin içeren yeni aril nitro-sülfonat hibrit bileşiğinin sentezi

Özellikle, yeni 2-(((4-nitrophenyl)imino)methyl)phenyl 2,4,6-trimethylbenzenesulfonate bileşiğinin trosinaz ve pankreatik lipaz enzimleri ile etkileşiminde standart olarak kullanılan kojic asit ve orlistat'a göre daha iyi bir skor elde edildiği gözlenmiştir. Asetilkolinesteraz ile yapılan çalışmada ise bağlanma affinite değeri standart olan donepezile (-11.6 kcal/mol) yakın bir değer olduğu gözlenmiştir. Yeni 2-(((4-nitrophenyl)imino)methyl)phenyl 2,4,6-trimethylbenzenesulfonate bileşiğinin in silico yöntem kullanılarak yapılan enzim inhibisyon çalışmasında bazı enzimlerde standartlara yakın bazılarında ise standartlardan daha iyi bir bağlanma affinitesi gösterdiği tespit edilmiştir. Bu kapsamda yeni sentezlenen 2-(((4-nitrophenyl)imino)methyl)phenyl 2,4,6-trimethylbenzenesulfonate bileşiğinin ileri

çalışmalarında yeni türevleri sentezlenerek bu bileşiklerin in vitro ve in vivo çalışmaları yapılmasıyla biyolojik özellikleri bakımından bilime katkı sunması beklenmektedir.

Anahtar Kelime: Azometin, Enzim inhibisyonu, aril nitro-sülfonat bileşikleri, Asetilkolinesteraz, pankreatik lipaz

SYNTHESIS, CHARACTERIZATION, AND MOLECULAR DOCKING STUDIES OF THE NOVEL ARYL NITRO-SULFONATE HYBRID COMPOUND BEARING AZOMETHINE

ABSTRACT

A novel 2-(((4-nitrophenyl)imino)methyl)phenyl 2,4,6-trimethylbenzenesulfonate was synthesized under mild conditions from 2,4,6-trimethylbenzenesulfonyl chloride and 2-(((4-nitrophenyl)imino)methyl)phenol which is prepared Dean-Stark method (Figure 1). In addition, 2-(((4-nitrophenyl)imino)methyl)phenyl 2,4,6-trimethylbenzenesulfonate was characterized by ^{1}H NMR and ^{13}C NMR. The enzyme inhibition of this compound with acetylcholinesterase, butyrylcholinesterase, glutathione s-transferase, tyrosinase, and pancreatic lipase was studied using in silico method. Molecular docking scores of the novel 2-(((4-nitrophenyl)imino)methyl)phenyl 2,4,6-trimethylbenzenesulfonate with acetylcholinesterase, butyrylcholinesterase, glutathione s-transferase, tyrosinase, and pancreatic lipase enzymes were found -11.0, -5.2, -6.7, - 7.8, and -9.8 kcal/mol, respectively.

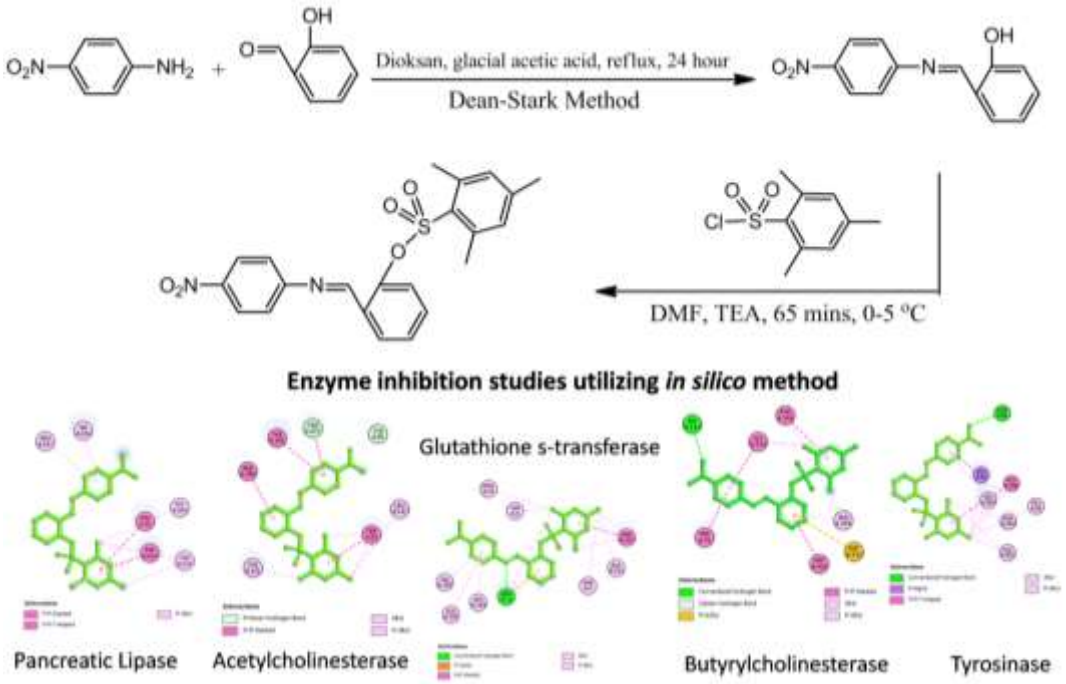


Figure 1. Synthesis of the novel aryl nitro-sulfonate hybrid compound bearing azomethine

In particular, it was observed that a better score was obtained in the interaction of the novel 2-(((4-nitrophenyl)imino)methyl)phenyl 2,4,6-trimethylbenzenesulfonate with tyrosinase and pancreatic lipase enzymes compared to kojic acid and orlistat as standards. In the study performed with acetylcholinesterase, it was observed that the binding affinity value was close to the standard donepezil (-11.6 kcal/mol). The enzyme inhibition study of the novel 2-(((4-nitrophenyl)imino)methyl)phenyl 2,4,6-trimethylbenzenesulfonate utilizing in silico method showed a binding affinity close to the standards in some enzymes or better than the standards

in others. In this context, it is expected that 2-(((4-nitrophenyl)imino)methyl)phenyl 2,4,6-trimethylbenzenesulfonate compound, which shows such biological properties, will contribute to science with new derivatives in future studies.

Keywords: Azomethine, enzyme inhibition, aryl nitro-sülfonate hybrid compounds, acetylcholinesterase, pancreatic lipase, tyrosinase.

YÜZEY GÖRÜNÜMÜ İYİLEŞTİRİLMİŞ YARI AROMATİK POLİAMİD 6 KARIŞIMLARININ ELDESİ

OBTAINING IMPROVED SURFACE APPEARANCE OF SEMI AROMATIC POLYAMIDE 6 BLENDS

(Araştırma ve İnovasyon Mühendisi), İbrahim Berk ÇAVUŞ
Orcid no: 0000-0001-8834-3101
Bursa Teknik Üniversitesi, Mühendislik ve Doğa Bilimleri Fakültesi

ÖZET

Poliamidler, otomotiv, elektrik-elektronik, beyaz eşya, ulaşım ve son tüketici ürünleri gibi çok farklı alanlarda sıklıkla tercih edilmektedir. Ticari alanda en sık kullanılan poliamid türleri poliamid (PA) 6 ve 6.6'dır. Ayrıca sektörde yüksek performanslı poliamid olarak da bilinen aromatik gruplara sahip polifitalamid'de (PPA) sıklıkla kullanılmaktadır. PPA, yapısında bulunan aromatik gruplar sayesinde yüksek mekanik özellik, yüksek bozunma sıcaklığı, yüksek boyutsal kararlılık, düşük nem absorbsiyonu gibi üstünlüklere sahiptir. Avantajlarının yanı sıra alifatik poliamidlere göre yüksek yoğunluk, yüksek erime sıcaklığı ve yüksek maliyet gibi dezavantajları da bulunmaktadır. Bu dezavantajların üstesinden gelmek için yarı aromatik poliamidlerin kullanımı önerilmektedir. Genel olarak, yarı aromatik poliamidlerin mekanik özellikleri, aromatik ve alifatik poliamidlerin sahip olduğu özellik değerlerinin arasında yer almaktadır. Bu çalışmada, %50 cam elyaf katkılı yarı aromatik poliamid ve alifatik poliamid karışımları hazırlanarak mekanik ve görsel anlamda polifenilen sülfit (PPS) yerine kullanılabilirliği araştırılmıştır. Elde edilen yeni ürünün modül değerinin 17000-18000 MPa, kopma mukavemeti> 235 MPa ve uzama değerleri> %2'den olduğu belirlenmiştir. Yapılan analizler sonucunda üretilen karışımların mekanik özellikleri, yoğunlukları, görsel yüzeyleri karşılaştırılmış olup, elde edilen ürünün maliyet ve özellik anlamında, PPS yerine kullanılabilir olduğu görülmüştür. Bunlara ek olarak, yeni ürünün gıda ve su ile temasa uygun olup, beyaz eşya ve mutfak aletleri gibi kısımlarda kullanılması önerilebilir.

Anahtar Kelimeler: yarı aromatik poliamid, alifatik poliamid, yüksek mekanik özellik, iyileştirilmiş yüzey görünümü

ABSTRACT

Polyamides are frequently preferred in many different areas such as automotive, electrical-electronics, white goods, transportation, and end-consumer products. The most used types of polyamides in the commercial field are polyamide (PA) 6 and 6.6. Polyphthalamide (PPA) is also most known polyamide type, and it is frequently used in the industry. Thanks to the aromatic groups in its structure, it has advantages such as high mechanical properties, high decomposition temperature, high dimensional stability, and low moisture absorption. it also has disadvantages such as high density, high melting temperature and cost compared to aliphatic polyamides. To overcome these disadvantages, the use of semi-aromatic polyamides is recommended. In general, the mechanical properties of semi-aromatic polyamides are among the properties of aromatic and aliphatic polyamides. In this study, it is planned to prepare 50% glass fiber reinforced semi-aromatic polyamide and aliphatic polyamide blends and their usability as mechanical and visual substitutes for polyphenylene sulfide (PPS) was investigated. It was determined that the modulus value of the new product obtained was 17000-18000 MPa, tensile strength> 235 MPa, and elongation values> 2%. As a result of the

analysis, the mechanical properties, densities, and visual surfaces of the mixtures produced were compared and it was seen that the product obtained could be used instead of PPS in terms of cost and properties. In addition, the new product is suitable for food and water contact, and it can be recommended to be used in parts such as white goods and kitchen appliances.

Keywords: semi aromatic polyamide, aliphatic polyamid, high mechanical properties, improved surface appaerance.

İÇİ BOŞ POLİBÜTİLEN SÜKSİNAT (PBS)/TERMOPLASTİK POLİÜRETAN (TPU) NANOLİFLERİN ÜRETİMİ VE KARAKTERİZASYONU

MSc. Hatice Bilge İŞGEN
ORCID: 0000-0002-6201-5836
Kocaeli University, Institute of Science, Polymer Science and Technology
Dr. Sema SAMATYA YILMAZ
ORCID: 0000-0002-2682-2892
Kocaeli University, Institute of Science, Polymer Science and Technology
Prof. Dr. Ayşe AYTAÇ
ORCID: 0000-0002-9566-7881
Kocaeli University, Institute of Science, Chemical Engineering, Polymer Science and Technology

ÖZET
Bu çalışmada, eş-zamanlı elektroçekim tekniği ile geliştirilmiş mekanik özelliklere sahip içi boş Polibütilen Süksinat (PBS)/Termoplastik Poliüretan (TPU) elektrospun matlar elde edilmiştir. Saf PBS, saf TPU ve PBS/TPU karışımlarının (60/40, 50/50, 40/60 w/w) çözeltileri, iki bileşenli nanofiberlerin kabuğuna beslenmiştir. Saf PVP çözeltisi ise iki bileşenli nanoliflerin çekirdeğine yönlendirildi. Daha sonra, iki bileşenli nanoliflerin çekirdeği, saf etanol çözücü içinde matların bekletilmesi ile yapıdan çıkarıldı. Sonuç olarak, içi boş elektrospun matlar elde edilmiş ve karakterizasyon testleri gerçekleştirilmiştir. Elde edilen matların karakterizasyonu için polarize optik mikroskop (POM), termogravimetrik analiz (TGA), mekanik analiz, Sıvı Emme Kapasitesi (LAC %) ve Kuruma Süresi Testi yapılmıştır. POM analizinde elektrospun matların morfolojik yapılarında, kısmen boncuklu fakat genellikle pürüzsüz nanolifler gözlendi. Özellikle, içi boş PBS/TPU (60/40) nanoliflerin en pürüzsüz nanolifler olduğu belirlenmiştir. TGA analizi ile içi boş nanoliflerin termal özellikleri araştırılmış ve ayrıca bu analiz ile tüm nanoliflerin çekirdeğindeki PVP'nin uzaklaştırıldığı görülmüştür. Çekme testi sonuçlarına göre, karışımlarda TPU oranı artırıldığında çekme gerilmesinin arttığı belirlendi. Karışımlarda yüksek miktarda PBS kullanıldığında sıvı emme kapasitesinin iyileştirdiği gözlenmiştir. Buna ek olarak, TPU oranı arttıkça içi boş nanoliflerin kuruma süreleri azalmıştır. Sonuç olarak, biyomedikal uygulamalar için biyolojik olarak parçalanabilen ve nefes alabilen PBS/TPU yara örtülerinin üretimi önerilmektedir.
Anahtar Kelimeler: PBS/TPU, içi boş nanolif, eş-eksenli elektroçekim, yara örtüsü.

PRODUCTION AND CHARACTERIZATION OF HOLLOW POLYBUTYLENE SUCCINATE (PBS)/THERMOPLASTIC POLYURETHANE (TPU) NANOFIBERS

ABSTRACT
In this paper, the hollow Polybutylene Succinate (PBS)/Thermoplastic Polyurethane (TPU) electrospun mats having developed mechanical structure were obtained with a coaxial electrospinning process. The solutions of neat PBS, neat TPU, and PBS/TPU blends (60/40, 50/50, 40/60 w/w) were fed into the shell of bi-component nanofibers. Meanwhile, the solution of neat PVP was directed into the core of bi-component nanofibers. Next, the core of bi-component nanofibers was removed from the structure by waiting for the mats in pure

ethanol solvent. As a result, hollow electrospun mats were obtained and characterization tests were carried out. Polarized optical microscope (POM), thermogravimetric analysis (TGA), mechanical analysis, Liquid Absorption Capacity (LAC %), and drying time test were performed for the characterization of the mats obtained. Partially beaded but generally smooth nanofibers were observed in the morphological structures of electrospun mats in POM analysis. Hollow PBS/TPU (60/40) nanofibers were determined as the smoothest nanofibers. The thermal properties of hollow nanofibers were investigated by TGA analysis, and it was also seen that PVP in the core of all nanofibers was removed with this analysis. According to the tensile test results, it was determined that when the TPU ratio was increased in blends, the tensile strain was enhanced. It was seen that when the high amount of PBS in blends improved the LAC %. In addition, the drying times of hollow nanofibers decreased with increasing TPU ratio. In conclusion, the production of biodegradable and breathable PBS/TPU dressings for biomedical applications is recommended.

Keywords: PBS/TPU, hollow nanofiber, coaxial electrospinning, wound dressing.

DİPEPTİT TAŞIYAN YENİ METAKRİLAT HOMOPOLİMERİNİN SENTEZİ, KARAKTERİZASYONU VE DİELEKTRİK ÖZELLİKLERİ

SYNTHESIS, CHARACTERIZATION AND DIELECTRIC PROPERTIES OF NOVEL METHACRYLATE HOMOPOLYMER WITH DIPEPTIDE SIDE CHAIN

Dr. Eray ÇALIŞKAN
ORCID 0000-0003-2399-4100
Bingol University, Faculty of Science and Arts, Department of Chemistry

ÖZET

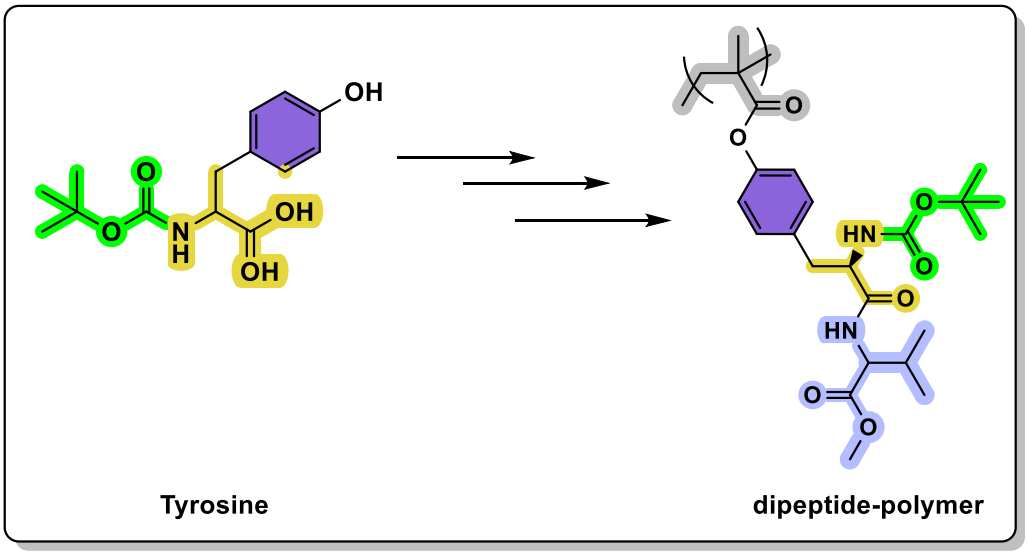
Bu çalışmada, amin ucu Boc korumalı Tirosin ile L-valin metil ester bileşiğinin reaksiyonu ile elde edilen dipepit bileşiğinin serbest fenolik OH kısmından metakrilat ile tepkimesi sonucu elde edilen yeni monomer üzerinden serbest radikalik polimerleşme yöntemi ile yeni bir homopolimer sentezlenmiştir. Bu polimerin impedans analiz cihazı ile dielektrik sabiti, dilelektrik kayıp faktörü ve ac iletkenlik parametreleri ölçülmüştür. Elde edilen sonuçlar literatürdeki benzer bileşikler ile kıyas edildiğinde bu polimerin göreceli olarak daha yüksek dilelektrik sabitine sahip olduğu tespit edilmiştir.

Anahtar Kelimeler: Dipeptit, polimer, dielektrik, iletkenlik, sentez

ABSTRACT

In this study, a new homopolymer was synthesized by the free radical polymerization method on the new monomer obtained as a result of the reaction of the dipeptide compound obtained by the reaction of amine end Boc-protected Tyrosine and L-valine methyl ester compound with methacrylate from the free phenolic OH part. The dielectric constant, dielectric loss factor, and ac conductivity parameters of this polymer were measured with an impedance analyzer. When the obtained results are compared with similar compounds in the literature, it has been determined that this polymer has a relatively higher dielectric constant.

Keywords: Dipeptide, polymer, dielectric, conductivity, synthesis



FENİTROTİYON'UN RATLARDA TESTİKULAR TOKSİSİTESİ ÜZERİNE GALLİK ASİT'İN KORUYUCU ROLÜ

PROTECTIVE ROLE OF GALLIC ACID ON FENITROTHION INDUCED TESTICULAR TOXICITY IN RATS

Enis Taha ÖZKAN
ORCID: 0000-0001-6248-4061
Gazi Üniversitesi, Fen Bilimleri Enstitüsü
Prof. Dr. Yusuf KALENDER
ORCID: 0000-0001-5457-0517
Gazi Üniversitesi, Fen Fakültesi

ÖZET

Fenitrothion, tarımsal zararlılara karşı sıklıkla kullanılan organofosfatlı insektisitlerden biridir. Gallik asit (3,4,5-trihidroksibenzoik asit), bazı bitkilerde doğal olarak oluşan fenolik bir bileşiktir. Pestisitler, hücrelerde ve dokularda reaktif oksijen türlerinin artmasına ve serbest radikallerin oluşmasına neden olmaktadır. Bu çalışmada, ratların testis dokusunda fenitrothion'un neden olabileceği histopatolojik değişiklikler, antioksidan enzim aktiviteleri ve malondialdehit (MDA) düzeylerindeki değişiklikler üzerine gallik asidin koruyucu etkisi araştırılmıştır. Ratlar dört gruba ayrılmıştır: 1. kontrol grubu, 2. gallik asit (50 mg/kg/gün) muameleli grup, 3. fenitrothion (32 mg/kg/gün; 1/25 LD_{50}) muameleli grup, 4. Fenitrothion (32 mg/kg/gün) ve gallik asit (50 mg /kg/gün) muameleli grup. Bu çalışma Gazi Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından onaylanmıştır (G.U. ET–19.025). Her grupta 6 rat bulunmaktadır. 28 günlük uygulamadan sonra fenitrothion uygulanan grup kontrol grubu ile karşılaştırıldığında, ratların testis dokularında MDA düzeyinde artış gözlenirken, antioksidan enzim aktivitelerinde [süperoksit dismutaz (SOD), katalaz (CAT), glutatyon-S-transferaz (GST), glutatyon peroksidaz (GPx)] istatistiksel olarak anlamlı bir azalma gözlendi. Fenitrothion muameleli grup, fenitrothion ve gallik asit muameleli grup ile karşılaştırıldığında, MDA seviyelerinde istatistiksel olarak anlamlı bir düşüş gözlenirken, SOD, CAT, GPX ve GST enzim aktivitelerinde istatistiksel olarak anlamlı bir artış gözlendi. Histopatolojik incelemelerde fenitrothion rat testislerinde spermatojenik hücre sayısında azalmaya, bazal membranın bozulmasına ve bazı seminifer tüpçüklerinde nekroza neden olmuştur. Gallik asit rat testislerindeki patolojik değişiklikleri azaltmıştır.

Anahtar Kelimeler: Fenitrotiyon, Gallik asit, Histopatoloji, Oksidatif stres.

ABSTRACT

Fenitrothion is one of the organophosphate insecticides frequently used against agricultural pests. Gallic acid (3,4,5-trihydroxybenzoic acid) is a naturally occurring phenolic compound in some plants. Pesticides cause an increase in reactive oxygen species in cells and tissues and the formation of free radicals. In this study, the protective effect of gallic acid on the histopathological changes, antioxidant enzyme activities and changes in malondialdehyde (MDA) levels that can be caused by fenithrothion in testicular tissue of rats were investigated. Rats were divided into four groups: 1. control group; 2. gallic acid (50 mg/kg/day) treated group 3. fenitrothion (32 mg/kg/day; 1/25 LD_{50}) treated group, 4. Fenitrothion (32 mg/kg/day) plus gallic acid (50 mg/kg/day) treated group. All procedures in this study were confirmed by Gazi University Animal Experiments Local Ethics Committe (G.U. ET–19.025). There are 6 rats in each group. After 28 days of treated, when the fenitrothion treated group was compared to the control group, an increase in the MDA level was observed in the testicular tissues of the rats, while antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx)] a statistically significant

decrease in was observed. When the fenitrothion treated group was compared with the fenitrothion plus gallic acid treated group, a statistically significant decrease was observed in the MDA levels, while a statistically significant increase was observed in the SOD, CAT, GPX and GST enzyme activities. In histopathological examinations, fenitrothion caused a decrease in the number of spermatogenic cells in the rat testes, disruption of the basement membrane and necrosis in some seminiferous tubules. Gallic acid reduced the pathological changes in rat testes.

Keywords: Fenitrothion, Gallic acid, Histopathology, Oxidative stress.

mTOR SİNYAL YOLAĞI VE KOLOREKTAL KANSER TEDAVİSİNDEKİ ROLÜ
mTOR SIGNALING PATHWAY AND ITS ROLE IN COLORECTAL CANCER TREATMENT

Dr. Öğretim Üyesi Şeyda BERK
ORCID: 0000-0003-4687-0223
Sivas Cumhuriyet Üniversitesi, Fen Fakültesi, Moleküler Biyoloji ve Genetik Bölümü

ÖZET
Kolorektal kanser (KRK), kanser ölümlerinin üçüncü nedeni olarak karşımıza çıkmaktadır ve dünya çapında en ölümcül insan malignitelerinden biridir. Bu kanser türü için farklı tedavi yöntemleri ile çeşitli cerrahi ve medikal tedaviler uygulanmaktadır. Kanser tedavisinde hastanın sağ kalımını ve yaşam kalitesini artırmak oldukça önemlidir ve bu alanda önemli gelişmeler olmasına rağmen kansere bağlı ölümlerde sürekli artışlar gözlenmektedir. Rapamisinin mekanik hedefi (mTOR), büyüme faktörü sinyallerini hücresel besin ve enerji seviyeleri ile bütünleştirerek hücre büyümesini, çoğalmasını ve hayatta kalmasını koordine etmektedir. Kolorektal kanser, yüksek insidans ve mortalite oranı nedeniyle en yoğun çalışılan kanser türürdür. Onkojenik Ras aktivasyonu, fosfatidilinositol 3-kinaz yolu hiperaktivasyonu, p53 mutasyonu ve Wnt sinyal yolunun düzensizliği dahil olmak üzere kolorektal karsinojenezde çoklu genetik değişiklikler rol oynamaktadır ve birçok onkojenik yol, mTOR yolunu aktive etmektedir. mTOR, son zamanlarda kolorektal kanser tedavisi için etkili bir hedef olarak ortaya çıkmıştır. mTOR yolunu hedefleyen *in vitro* ve preklinik çalışmalar KRK kemoterapisi için umut verici bakış açıları sağlamıştır. mTOR inhibitörünün sitotoksik kemoterapi, vasküler endotelyal büyüme faktörü (VEGF) inhibitörleri, epidermal büyüme faktörü reseptörü (EGFR) ve Mitojenle aktive olan protein kinaz kinaz (MEK) inhibitörleri gibi ajanlarla kombinasyon uygulamaları yoğun bir şekilde araştırılmakta birlikte umut verici sonuçlar elde edilmiştir. Optimize tedavi yöntemleri geliştirmek için mTOR sinyal ağındaki moleküler mekanizmanın daha fazla anlaşılması gerekmektedir. Rapamisinin memeli hedefi (mTOR), özellikle kanser biyolojisinde çok önemli bir rol oynaması nedeniyle ilaç geliştirme için potansiyel bir hedef olarak ortaya çıkmıştır ve yeni nesil mTOR inhibitörleri ortaya çıkmıştır. Tüm bu gelişmlere ışığında özellikle kolorekta kanser tedavisinde mTOR tabanlı terapi heyecan verici yeni bir aşamaya işaret etmektedir.
Anahtar Kelimeler: Kolorektal kanser, mTOR, rapamisin

ABSTRACT
Colorectal cancer (CRC) is the third cause of cancer death and is one of the deadliest human malignancies worldwide. Various surgical and medical treatments are applied with different treatment methods for this type of cancer. It is very important to improve the survival and quality of life of the patient in cancer treatment, and although there are important developments in this area, there is a continuous increase in cancer-related deaths. The mechanical target of rapamycin (mTOR) coordinates cell growth, proliferation, and survival by integrating growth factor signals with cellular nutrient and energy levels. Colorectal cancer is the most extensively studied cancer type due to its high incidence and mortality rate. Multiple genetic alterations are implicated in colorectal carcinogenesis, including oncogenic Ras activation, phosphatidylinositol 3-kinase pathway hyperactivation, p53 mutation, and dysregulation of the Wnt signaling pathway, and many oncogenic pathways activate the mTOR pathway. mTOR has recently emerged as an effective target for colorectal cancer therapy. *In vitro* and preclinical studies targeting the mTOR pathway have provided

promising perspectives for CRC chemotherapy. Combination applications of mTOR inhibitor with agents such as cytotoxic chemotherapy, vascular endothelial growth factor (VEGF) inhibitors, epidermal growth factor receptor (EGFR) and Mitogen-activated protein kinase kinase (MEK) inhibitors have been extensively investigated, but promising results have been obtained. Further understanding of the molecular mechanism in the mTOR signaling network is needed to develop optimized treatment modalities. The mammalian target of rapamycin (mTOR) has emerged as a potential target for drug development, especially because it plays a crucial role in cancer biology, and a new generation of mTOR inhibitors has emerged. In light of all these developments, mTOR-based therapy points to an exciting new stage, especially in colorectal cancer treatment.
Keywords: Colorectal cancer, mTOR, rapamycin.

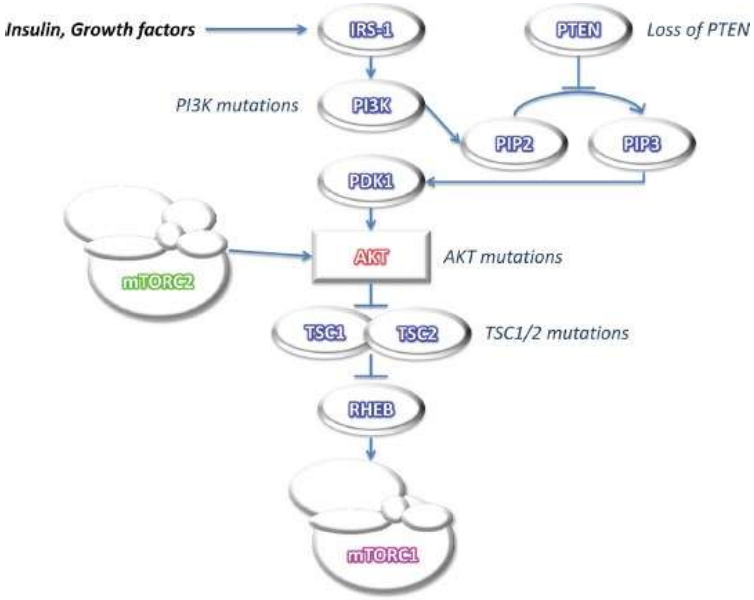
1. GİRİŞ

Kolorektal kanser (KRK), kanser ölümlerinin üçüncü nedeni olarak karşımıza çıkmaktadır ve dünya çapında en ölümcül insan malignitelerinden biridir (Inamura, 2018). Bu kanser türü için farklı tedavi yöntemleri ile çeşitli cerrahi ve medikal tedaviler uygulanmaktadır (Pellino ve ark., 2018). Kanser tedavisinde hastanın sağ kalımını ve yaşam kalitesini artırmak oldukça önemlidir ve bu alanda önemli gelişmeler olmasına rağmen kansere bağlı ölümlerde sürekli artışlar gözlenmektedir (Yadav ve ark., 2017). Rapamisinin mekanik hedefi (mTOR), büyüme faktörü sinyallerini hücresel besin ve enerji seviyeleri ile bütünleştirerek hücre büyümesini, çoğalmasını ve hayatta kalmasını koordine etmektedir (Francipane & Lagasse, 2014). Kolorektal kanser, yüksek insidans ve mortalite oranı nedeniyle en yoğun çalışılan kanser türürdür. Onkojenik Ras aktivasyonu, fosfatidilinositol 3-kinaz yolu hiperaktivasyonu, p53 mutasyonu ve Wnt sinyal yolunun düzensizliği dahil olmak üzere kolorektal karsinojenezde çoklu genetik değişiklikler rol oynamaktadır ve birçok onkojenik yol, mTOR yolunu aktive etmektedir (Francipane & Lagasse, 2014).

2. mTOR SİNYAL YOLAĞI

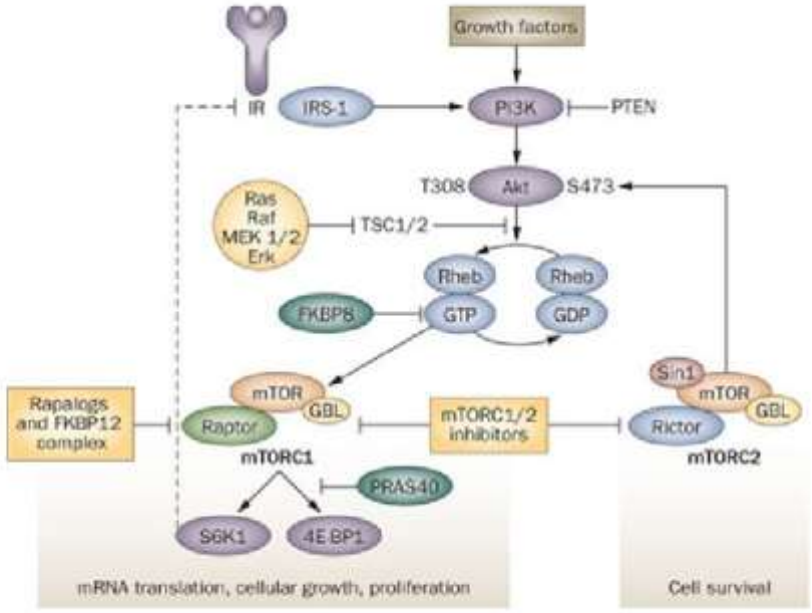
Rapamisinin memeli hedefi (mTOR), özellikle kanser biyolojisinde çok önemli bir rol oynaması nedeniyle ilaç geliştirme için potansiyel bir hedef olarak ortaya çıkmıştır. Ayrıca, yeni nesil mTOR inhibitörleri ortaya çıkmıştır ve bu gelişim, mTOR tabanlı terapide umut vaad eden gelişmelere neden olmuştur. Ancak, terapötik etkinlikleri hakkındaki karar belirsizliğini korumaktadır ve bunun aydınlatılması için klinik ve deneysel çalışmalar devam etmektedir.

Klasik olarak, Akt, rapamisinin memeli hedefinin (mTOR) ana upstream aktivatörü olarak görev almaktadır (Şekil 1). Aktive edilmiş Akt, tüberoskleroz 2'yi (TSC2) fosforile eder ve inhibe eder, beyinde zenginleştirilmiş Ras homologunun (Rheb) GTP'ye bağlı durumda birikmesine ve mTOR kompleksi1 (mTORC1) yolunun aktivasyonunu tetiklemesine izin verir (Şekil 1). mTORC1, mTOR'un düzenleyici ilişkili proteini (Raptor), mLST8/G-protein β-alt birimi benzeri protein (GβL), RAS40 ve Deptor olan mTOR'dan oluşur (Şekil 2). mTORC1'de mTOR'un aktivasyonu, protein translasyonu ve hücre büyümesinin aracıları olan ribozomal S6 protein kinaz 1 (S6K1) ve eIF4E bağlayıcı protein 1 (4E-BP1) fosforilasyonuna yol açar (Fingar ve ark., 2002) (Şekil 2).



Şekil 1. mTOR yolu aktivasyonunun basitleştirilmiş şeması. Akt aktivasyonu, Rheb'yi TSC1 / 2'nin inhibe edici etkilerinden serbest bırakır ve böylece mTOR aktivasyonuna izin verir. PI3K/Akt/mTOR yolundaki mTOR'un yukarısındaki sinyal kusurları (PTEN, PI3K, Akt ve TSC'lerdeki mutasyonlar) mTOR kuralsızlaştırmasına yol açar. Akt ayrıca, iki fonksiyonel olarak farklı sinyal kompleksi, mTORC1 ve mTORC2 oluşturmak için farklı protein ortaklarıyla mTOR ilişkisi nedeniyle mTOR'un aşağı akış efektörü olabilir (Francipane & Lagasse, 2014).

Çok çeşitli hücre içi (enerji ve stres) ve hücre dışı (besinler, büyüme faktörleri, hormonlar) sinyallere mTOR yanıtı bu efektörler aracılığıyla aracılık eder. Besin ve büyüme faktörü mevcudiyetine yanıt olarak, mTORC1, metabolik olarak stresli hücrelerin, hayatta kalmaları için gerekli enerjiyi geri kazanmak için organeller dahil sitoplazmik bileşenleri geri dönüştürdüğü bir süreç olan otofajiyi bastırır. mTORC1'in ayrıca yakın zamanda pirimidin sentez yolu yoluyla nükleotid sentezini uyararak anabolik hücre büyümesini düzenlediği de tanımlanmıştır (Ben-Sahra ve ark., 2013).



Şekil 2: mTOR sinyal yolları (Dancey, 2010).

mTORC1'den farklı olarak mTORC2, mTOR, mTOR'un rapamisine duyarsız eşlikçisi (Rictor), mLST8/GβL, stresle aktive olan protein kinaz ile etkileşime giren protein 1 (Sin1), prolin bakımından zengin tekrar protein-5'ten (PRR-5) oluşur.)/protein, Rictor-1 (Protor-1) ve Deptor ile gözlemlendi. mTORC2'nin upstream düzenlemesi iyi tanımlanmamıştır, ancak ribozom ilişkisi mTORC2 aktivasyonunun tek olmasa da ana mekanizması gibi görünmektedir (Zinzalla ve ark., 2011). mTORC2, Akt'nin tam aktivasyonuna izin vererek, Protein Kinaz Ca (PKCα), Serum/glukokortikoidle düzenlenen kinazı 1 (SGK1) ve Akt'yi fosforile ettiği için hücre sağkalımı, metabolizması, çoğalması ve hücre iskeleti organizasyonunda önemli bir rol oynar (García-Martínez & Alessi, 2008; Jacinto ve ark., 2004; Sarbassov ve ark., 2004; Sarbassov ve ark., 2005). Bu nedenle Akt, hem mTORC1'in yukarı akış aktivatörü hem de mTORC2'nin aşağı akış efektörüdür (Şekil 1 ve 2).

Kanser biyolojisinde çok önemli bir rol oynadığı için mTOR, ilaç geliştirme için potansiyel bir hedef olarak ortaya çıkmıştır. Birkaç mTOR inhibitörü, çeşitli kanserleri tedavi etmek için büyük bir başarı olmadan klinik deneylerden geçmiştir. Bununla birlikte, yeni bileşikler sentezlendikçe mTOR inhibitörlerinin kanser tedavisindeki rolü gelişmeye devam etmektedir.

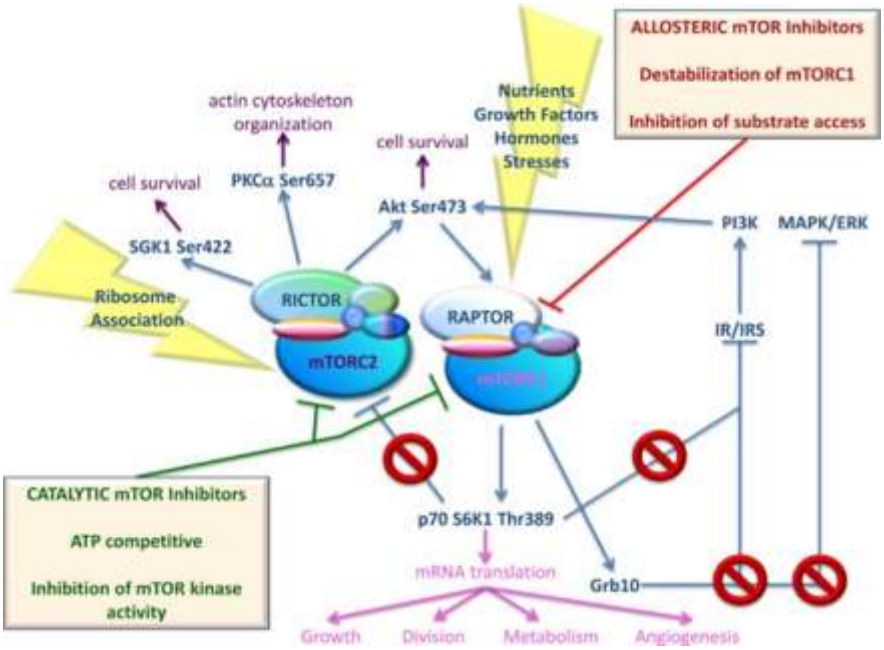
mTOR, insan kanserlerinde sıklıkla aktive olmasına rağmen, mTOR geninin mutasyonu sadece nediren gözlemlenmiştir (Robbins ve ark., 2011). Bu, mTOR yolunun aşırı aktivasyonunun çoğunlukla fosfatidilinositol-3-kinaz (PI3K)/Akt/mTOR yolundaki mTOR'un upstream sinyal kusurlarından kaynaklandığı anlamına gelmektedir. PI3K alfa katalitik alt birim kinaz alanındaki (PIK3CA) mutasyonlar genellikle tümörijenezde geç ortaya çıkar ve CRC tümörlerinin %32'sinde tanımlanabilmektedir (Samuels ve ark., 2004). PI3K aktivitesinin negatif düzenleyicisi olan Fosfataz ve tensin homologunda (PTEN) heterozigosite kaybı (LOH) ve mutasyonlar da CRC'de rapor edilmiştir. Hem PIK3CA mutasyonları hem de PTEN kaybı, mTOR aşırı aktivasyonuna yol açar (Zhou ve ark., 2002).

Akt genlerindeki mutasyonlar CRC'de nadiren bulunsa da, pleckstrin homoloji (PH) alanındaki Akt1'in (E17K) somatik bir yanlış anlamlı mutasyonu, Akt1'in plazma zarı ile yapıcı ilişkisine ve CRC'de Akt1'in uzun süreli aktivasyonuna neden olur. mTOR kuralsızlaştırmasına yol açar (Carpten ve ark., 2007; Soung ve ark., 2006). Benzer şekilde,

nadir olmakla birlikte, kolonik hamartomatöz poliplerle ilişkili olan germline TSC gen mutasyonları, muhtemelen mTOR yolu tetiklemesi yoluyla %1 CRC'den sorumludur (Şekil 1) (Devroede ve ark., 1988; Zbuk & Eng, 2007).

3. mTOR İNHİBİTÖRLERİ

Streptomyces hygroscopicus tarafından üretilen bir makrolid antibiyotik olan rapamisin, keşfedilen ilk mTOR inhibitörüdür . Daha doğrusu, araştırmacılar Rapamisin'in mantar önleyici özelliklerinden yararlanarak mTOR'u tanımlayabilmişlerdir (Heitman ve ark., 1991). Guba ve arkadaşları 2002 yılında Nature Medicine makalelerinde Rapamisin antitümör etkilerini ilk kez tanımladılar (Guba ve ark., 2002). Geleneksel olarak organ nakli için bir immünosupresan olarak kullanılan Rapamisinin, anjiyogenezi inhibe ederek tümör büyümesini baskıladığı bulunmuştur (Şekil 3). O zamandan beri, Rapalog ailesini oluşturan Rapamisin'e benzer özelliklere sahip ek mTOR işlevi inhibitörleri sentezlenmiştir. Everolimus, Temsirolimus ve Ridaforolimus'u da içeren rapaloglar, FKBP12'yi bağlar ve mTOR'un FRB alanına müdahale etmektedir. Bu bileşikler, çeşitli kanserleri tedavi etmek için tek ajanlar olarak klinik deneylerden büyük bir başarı elde edememiştir. Bununla birlikte, Temsirolimus ve Everolimus'un her ikisi de ileri böbrek hücreli karsinom (RCC) üzerinde etkili olarak bulunmuştur (Battelli & Cho, 2011).



Şekil 3. Farklı mTOR inhibitörleri kullanılarak mTOR inhibisyonunun etkileri ile birlikte her iki mTOR kompleksinin aktivatörlerinin ve efektörlerinin basitleştirilmiş şeması (Francipane & Lagasse, 2014)

Rapaloglara direnç, mayalarda FKBP12 veya TOR'un FKB alanındaki mutasyonlarla ilişkilendirilirken (Lorenz & Heitman, 1995), insanda çoğunlukla KRAS ve BRAF mutasyonları ile ilişkilendirilmiştir (Mohseni & Park, 2010). Ek direnç mekanizmaları, onkojenik serin/treonin kinazların Moloney murin lösemi virüsü (PIM) ailesi için Proviral entegrasyon bölgesinin yukarı regülasyonunu, oksidatif stresi ve anti-apoptotik proteinlerin aşırı ekspresyonunu içerir (Şekil 3) (Fox ve ark., 2005; Majumder ve ark., 2004; Neklesa & Davis, 2008).

4. Kolonda mTOR sinyali

mTORC1, organizmanın beslenme durumunun önemli bir sensörüdür. Gerçekten de, kalori kısıtlaması, memeli bağırsak kök hücre (ISC) nişinin önemli bir bileşeni olan Paneth hücrelerinde mTORC1 sinyalini düşürür. Paneth hücreleri, sırayla, küçük bağırsak kök

hücrelerini çoğalmaya teşvik eder (Majumder ve ark., 2004). Böylece, mTOR inhibisyonu, bağırsak atrofisinden etkilenen hastalarda bağırsak yenilenmesini iyileştirebilir. mTORC1 inhibisyonu ISC'lerin sayısını ve rejeneratif kapasitesini artırabilirken, aşırı mTORC1 stimülasyonu kanserin başlamasına neden olabilir. Gerçekten de, bağırsak polip oluşumunda mTORC1 yolunun önemi, ailesel adenomatöz polipozun (FAP) bir fare modeli olan ApcΔ716 farelerinin kullanıldığı birkaç çalışmada iyi tanımlanmıştır. Bu çalışmalar sayesinde, mTORC1'in anafaz köprüsü oluşumu yoluyla kromozomal kararsızlığı (CIN) uyardığı ve bunun sonucunda hem tümörün başlamasını hem de ilerlemesini arttırdığı bulunmuştur (Aoki ve ark., 2003). Benzer şekilde, insan kolorektal adenomları ve kanserleri üzerinde yapılan *ex vivo* immünohistokimyasal çalışmalar, mTORC1 sinyalinin tümörijenez sürecinde erken bir olay olarak meydana geldiğini ve normal hücrelerin neoplastik bir fenotipe ilerlemesine katıldığını ve kolon polipleri ve kanserlerinin tedavisi ve önlenmesi için mTORC1 hedefli ilaç geliştirmenin temellerini sürdürdüğünü doğrulanmıştır (Zhang ve ark., 2009). Buna göre, Everolimus aracılı mTORC1 inhibisyonu, ApcΔ716 farelerinde polip oluşumunu baskıladığı ve mortaliteyi azalttığı gözlemlenmiştir (Fujishita ve ark., 2008). Bununla birlikte, belirli bir yolun bloke edilmesi, sinyal yolları arasındaki dengeyi bozabilir ve onkojenik sinyalleri artırabilir. Bu bağlamda, sitostatik etkisine paralel olarak, Rapamisin tarafından mTORC1 inhibisyonu, MAPK kinaz (MEK)/ERK aktivitesini güçlü bir şekilde arttırdı, bu da bir iğ morfolojisinin ortaya çıkmasına ve KRAS ile dönüştürülmüş bağırsak epitel hücrelerinin (IEC'ler) daha yüksek istilasına neden olmuştur (Shao ve ark., 2004). Bu sistemde, Rapamisin tedavisi ayrıca Bcl-2 seviyelerini arttırmıştır, ayrıca hayatta kalma, pro-invaziv ve pro-metastatik yolların geri besleme inhibisyonunun hafifletilmesinin üstesinden gelebilecek yeni terapötik ilaçlar geliştirme ihtiyacına işaret etmiştir (Shao ve ark., 2004).

mTORC1'in yanı sıra, mTORC2'nin CRC'de aşırı eksprese edildiği ve kanser biyolojisinde önemli bir rol oynadığı gösterilmiştir. mTORC2'nin aşağı regülasyonu, kolon kanseri hücre hatlarının proliferasyonunu azaltmıştır ve *in vivo* olarak tümör ksenograftlarının oluşumunu inhibe etmiştir (Gulhati ve ark., 2009; Roulin ve ark., 2010). Ayrıca, hem mTORC1 hem de mTORC2 komplekslerinin, CRC'lerin epitelyal-mezenkimal geçişini (EMT), hareketliliğini ve metastazını RhoA ve Rac1 GTPazlar yoluyla düzenlediği gösterilmiştir ve bu da, ATP-rekabetçi mTOR seçici veya PI3K/mTOR ikili inhibitörlerini CRC hastalarının tedavisi için dahil etme gerekçesini sağlamıştır (Gulhati ve ark., 2011).

5. SONUÇ:

mTOR, insan kanserlerinde sıklıkla aktive edilir ve yaygın olarak aranan bir antikanser terapötik hedefidir. mTOR'un doğrudan substratları ve downstream efektörleri iyi çalışılmış olsa da, mTOR'un geniş fizyolojik rolü, hala tanımlanacak downstream yollarının olduğu anlamına gelmektedir. Bu nedenle, mTOR yolunu hedefleyen güvenli ve etkili ilaç moleküllerinin tasarımı için mTOR sinyal ağlarının dinamiklerinin daha kapsamlı bir şekilde anlaşılması gerekmektedir. ATP rekabetçi mTOR inhibitörlerinin hem avantajları hem de dezavantajları vardır. Kuşkusuz iyi tanımlanmış bir hedefleri vardır, biyolojik etkilerinin gözlemlenmesi kolaydır ve hızlı bir şekilde klinik deneylere geçerler. Öte yandan, genellikle diğer kinazlarla yüksek düzeyde çapraz reaktiviteye sahiptirler, farmakolojik olarak tolere edilmezler ve kolayca ilaç direncinin gelişmesine yol açarlar. Etkinliği veya mTOR inhibitörlerine karşı direnci öngörebilecek biyolojik faktörlerin belirlenmesi, mTOR inhibitörleri, birden fazla etki mekanizması yoluyla antitümör etkileri gösterebileceğinden, hala zordur. Ayrıca, mTOR yolunun diğer yollarla karışması, tümör hücrelerinin anti-tümör uyaranlarından kaçması için telafi edici döngülere yol açmaktadr. Bu, onkojenik sinyal ağlarının uyarlanabilir yeteneklerini ve geri besleme ile düzenlenen yolları inhibe etmek için monoterapinin sınırlamalarını ortaya koymaktadır. Bu direncin üstesinden gelmek için birden fazla yolu hedefleyen ilaç kombinasyonlarından yararlanılmıştır, ancak hangi stratejinin daha fazla terapötik fayda sağlayacağı hala açık değildir.

Sonuç olarak, hedefe yönelik tedavi ajanları klinik uygulamalar için giderek daha fazla kullanılabilir olmasına rağmen, bu umut verici ilaçların çoğu klinik deneylerde test edildiğinde başarılı sonuçlar elde edilememiştir. Çoğu tümör heterojen olduğundan, aynı tümör tipine/histolojisine sahip hastalar için tek bir ilaç rejimi her zaman uygun değildir. Gen ve protein imzaları çeşitli hastalıklarda tanımlanmıştır, ancak bu bilgi klinik olarak anlamlı gelişmelere dönüştürülmek için mücadele etmektedir.
Bununla birlikte, bu gözlemler, yakın gelecekte umut verici bir şekilde, bireyselleştirilmiş ilaç tedavisine ulaşma zorluğunun üstesinden gelmeye yardımcı olabilir.

KAYNAKÇA

Aoki, K., Tamai, Y., Horiike, S., Oshima, M., & Taketo, M. M. (2003). Colonic polyposis caused by mTOR-mediated chromosomal instability in Apc+/Delta716 Cdx2+/- compound mutant mice. *Nat Genet*, *35*(4), 323-330. https://doi.org/10.1038/ng1265

Battelli, C., & Cho, D. C. (2011). mTOR inhibitors in renal cell carcinoma. *Therapy*, *8*(4), 359-367. https://doi.org/10.2217/thy.11.32

Ben-Sahra, I., Howell, J. J., Asara, J. M., & Manning, B. D. (2013). Stimulation of de novo pyrimidine synthesis by growth signaling through mTOR and S6K1. *Science*, *339*(6125), 1323-1328. https://doi.org/10.1126/science.1228792

Carpten, J. D., Faber, A. L., Horn, C., Donoho, G. P., Briggs, S. L., Robbins, C. M., Hostetter, G., Boguslawski, S., Moses, T. Y., Savage, S., Uhlik, M., Lin, A., Du, J., Qian, Y. W., Zeckner, D. J., Tucker-Kellogg, G., Touchman, J., Patel, K., Mousses, S., . . . Thomas, J. E. (2007). A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature*, *448*(7152), 439-444. https://doi.org/10.1038/nature05933

Dancey, J. (2010). mTOR signaling and drug development in cancer. *Nature Reviews Clinical Oncology*, *7*(4), 209-219. https://doi.org/10.1038/nrclinonc.2010.21

Devroede, G., Lemieux, B., Massé, S., Lamarche, J., & Herman, P. S. (1988). Colonic hamartomas in tuberous sclerosis. *Gastroenterology*, *94*(1), 182-188. https://doi.org/10.1016/0016-5085(88)90628-2

Fingar, D. C., Salama, S., Tsou, C., Harlow, E., & Blenis, J. (2002). Mammalian cell size is controlled by mTOR and its downstream targets S6K1 and 4EBP1/eIF4E. *Genes Dev*, *16*(12), 1472-1487. https://doi.org/10.1101/gad.995802

Fox, C. J., Hammerman, P. S., & Thompson, C. B. (2005). The Pim kinases control rapamycin-resistant T cell survival and activation. *J Exp Med*, *201*(2), 259-266. https://doi.org/10.1084/jem.20042020

Francipane, M. G., & Lagasse, E. (2014). mTOR pathway in colorectal cancer: an update. *Oncotarget*, *5*(1), 49-66. https://doi.org/10.18632/oncotarget.1548

Fujishita, T., Aoki, K., Lane, H. A., Aoki, M., & Taketo, M. M. (2008). Inhibition of the mTORC1 pathway suppresses intestinal polyp formation and reduces mortality in ApcDelta716 mice. *Proc Natl Acad Sci U S A*, *105*(36), 13544-13549. https://doi.org/10.1073/pnas.0800041105

García-Martínez, J. M., & Alessi, D. R. (2008). mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). *Biochem J*, *416*(3), 375-385. https://doi.org/10.1042/bj20081668

Guba, M., von Breitenbuch, P., Steinbauer, M., Koehl, G., Flegel, S., Hornung, M., Bruns, C. J., Zuelke, C., Farkas, S., Anthuber, M., Jauch, K. W., & Geissler, E. K. (2002). Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat Med*, *8*(2), 128-135. https://doi.org/10.1038/nm0202-128

Gulhati, P., Bowen, K. A., Liu, J., Stevens, P. D., Rychahou, P. G., Chen, M., Lee, E. Y., Weiss, H. L., O'Connor, K. L., Gao, T., & Evers, B. M. (2011). mTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways. *Cancer Res*, *71*(9), 3246-3256. https://doi.org/10.1158/0008-5472.Can-10-4058

Gulhati, P., Cai, Q., Li, J., Liu, J., Rychahou, P. G., Qiu, S., Lee, E. Y., Silva, S. R., Bowen, K. A., Gao, T., & Evers, B. M. (2009). Targeted inhibition of mammalian target of rapamycin signaling inhibits tumorigenesis of colorectal cancer. *Clin Cancer Res*, *15*(23), 7207-7216. https://doi.org/10.1158/1078-0432.Ccr-09-1249

Heitman, J., Movva, N. R., & Hall, M. N. (1991). Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science*, *253*(5022), 905-909. https://doi.org/10.1126/science.1715094

Inamura, K. (2018). Colorectal Cancers: An Update on Their Molecular Pathology. *Cancers (Basel)*, *10*(1). https://doi.org/10.3390/cancers10010026

Jacinto, E., Loewith, R., Schmidt, A., Lin, S., Rüegg, M. A., Hall, A., & Hall, M. N. (2004). Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol*, *6*(11), 1122-1128. https://doi.org/10.1038/ncb1183

Lorenz, M. C., & Heitman, J. (1995). TOR mutations confer rapamycin resistance by preventing interaction with FKBP12-rapamycin. *J Biol Chem*, *270*(46), 27531-27537. https://doi.org/10.1074/jbc.270.46.27531

Majumder, P. K., Febbo, P. G., Bikoff, R., Berger, R., Xue, Q., McMahon, L. M., Manola, J., Brugarolas, J., McDonnell, T. J., Golub, T. R., Loda, M., Lane, H. A., & Sellers, W. R. (2004). mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. *Nat Med*, *10*(6), 594-601. https://doi.org/10.1038/nm1052

Mohseni, M., & Park, B. H. (2010). PIK3CA and KRAS mutations predict for response to everolimus therapy: now that's RAD001. *J Clin Invest*, *120*(8), 2655-2658. https://doi.org/10.1172/jci44026

Neklesa, T. K., & Davis, R. W. (2008). Superoxide anions regulate TORC1 and its ability to bind Fpr1:rapamycin complex. *Proc Natl Acad Sci U S A*, *105*(39), 15166-15171. https://doi.org/10.1073/pnas.0807712105

Pellino, G., Warren, O., Mills, S., Rasheed, S., Tekkis, P. P., & Kontovounisios, C. (2018). Comparison of Western and Asian Guidelines Concerning the Management of Colon Cancer. *Dis Colon Rectum*, *61*(2), 250-259. https://doi.org/10.1097/dcr.0000000000001012

Robbins, C. M., Tembe, W. A., Baker, A., Sinari, S., Moses, T. Y., Beckstrom-Sternberg, S., Beckstrom-Sternberg, J., Barrett, M., Long, J., Chinnaiyan, A., Lowey, J., Suh, E., Pearson, J. V., Craig, D. W., Agus, D. B., Pienta, K. J., & Carpten, J. D. (2011). Copy number and targeted mutational analysis reveals novel somatic events in metastatic prostate tumors. *Genome Res*, *21*(1), 47-55. https://doi.org/10.1101/gr.107961.110

Roulin, D., Cerantola, Y., Dormond-Meuwly, A., Demartines, N., & Dormond, O. (2010). Targeting mTORC2 inhibits colon cancer cell proliferation in vitro and tumor formation in vivo. *Mol Cancer*, *9*, 57. https://doi.org/10.1186/1476-4598-9-57

Samuels, Y., Wang, Z., Bardelli, A., Silliman, N., Ptak, J., Szabo, S., Yan, H., Gazdar, A., Powell, S. M., Riggins, G. J., Willson, J. K., Markowitz, S., Kinzler, K. W., Vogelstein, B., & Velculescu, V. E. (2004). High frequency of mutations of the PIK3CA gene in human cancers. *Science*, *304*(5670), 554. https://doi.org/10.1126/science.1096502

Sarbassov, D. D., Ali, S. M., Kim, D. H., Guertin, D. A., Latek, R. R., Erdjument-Bromage, H., Tempst, P., & Sabatini, D. M. (2004). Rictor, a novel binding partner of mTOR,

defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol*, *14*(14), 1296-1302. https://doi.org/10.1016/j.cub.2004.06.054

Sarbassov, D. D., Guertin, D. A., Ali, S. M., & Sabatini, D. M. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science*, *307*(5712), 1098-1101. https://doi.org/10.1126/science.1106148

Shao, J., Evers, B. M., & Sheng, H. (2004). Roles of phosphatidylinositol 3'-kinase and mammalian target of rapamycin/p70 ribosomal protein S6 kinase in K-Ras-mediated transformation of intestinal epithelial cells. *Cancer Res*, *64*(1), 229-235. https://doi.org/10.1158/0008-5472.can-03-1859

Soung, Y. H., Lee, J. W., Nam, S. W., Lee, J. Y., Yoo, N. J., & Lee, S. H. (2006). Mutational analysis of AKT1, AKT2 and AKT3 genes in common human carcinomas. *Oncology*, *70*(4), 285-289. https://doi.org/10.1159/000096289

Yadav, N. K., Arya, R. K., Dev, K., Sharma, C., Hossain, Z., Meena, S., Arya, K. R., Gayen, J. R., Datta, D., & Singh, R. K. (2017). Alcoholic Extract of Eclipta alba Shows In Vitro Antioxidant and Anticancer Activity without Exhibiting Toxicological Effects. *Oxid Med Cell Longev*, *2017*, 9094641. https://doi.org/10.1155/2017/9094641

Zbuk, K. M., & Eng, C. (2007). Hamartomatous polyposis syndromes. *Nat Clin Pract Gastroenterol Hepatol*, *4*(9), 492-502. https://doi.org/10.1038/ncpgasthep0902

Zhang, Y. J., Dai, Q., Sun, D. F., Xiong, H., Tian, X. Q., Gao, F. H., Xu, M. H., Chen, G. Q., Han, Z. G., & Fang, J. Y. (2009). mTOR signaling pathway is a target for the treatment of colorectal cancer. *Ann Surg Oncol*, *16*(9), 2617-2628. https://doi.org/10.1245/s10434-009-0555-9

Zhou, X. P., Loukola, A., Salovaara, R., Nystrom-Lahti, M., Peltomäki, P., de la Chapelle, A., Aaltonen, L. A., & Eng, C. (2002). PTEN mutational spectra, expression levels, and subcellular localization in microsatellite stable and unstable colorectal cancers. *Am J Pathol*, *161*(2), 439-447. https://doi.org/10.1016/s0002-9440(10)64200-9

Zinzalla, V., Stracka, D., Oppliger, W., & Hall, M. N. (2011). Activation of mTORC2 by association with the ribosome. *Cell*, *144*(5), 757-768. https://doi.org/10.1016/j.cell.2011.02.014

İKLİM DEĞİŞİKLİĞİNİN SULAK ALAN EKOSİSTEMLERİ ÜZERİNDEKİ ETKİSİ: YEŞİLIRMAK DELTASINDA SIĞ SULAK ALANLARDA BİR İNCELEME

IMPACT OF CLIMATE CHANGE ON WETLAND ECOSYSTEMS: AN INVESTIGATION IN SHALLOW WETLANDS IN YEŞILIRMAK DELTA

Dr. Halim TOPALDEMİR
Orcid no: 0000-0002-4494-9715
Ordu Üniversitesi, Fen Edebiyat Fakültesi
Prof. Dr. Beyhan TAŞ
Orcid no: 0000-0001-6421-2561
Ordu Üniversitesi, Fen Edebiyat Fakültesi

ÖZET

Son çeyrek asırdan itibaren belirgin hale gelen küresel iklim değişikliği tatlı su kaynaklarının azalmasına, kuraklık ve çölleşmenin yanı sıra ekosistem, tür ve genetik çeşitlilik kayıplarına yol açmaktadır. İklim değişikliği sulak alanlar için büyük bir tehdit olarak tanımlanmaktadır. Son yıllarda Türkiye'de değişen iklim koşullarına bağlı olarak, Akdeniz iklim bölgesinde aşırı kuraklık, Karadeniz iklim bölgesinde ise kuraklıkla beraber ani ve şiddetli yağışlar ve sel afetleri yaşanmaktadır. Bu çalışmada, Orta Karadeniz Bölümü'nde yer alan Yeşilırmak Deltasındaki (Terme, Samsun) bazı sulak alanlarda iklim değişikliğinin etkisi dört yıl boyunca gözlemlendi. Yağışlı (Kasım-Mart) ve kurak (Haziran-Eylül) sezonlarda yapılan incelemelerde, sucul ekosistemler için çok önemli fonksiyonları olan yaygın sucul bitkilerin teşhisleri de yapıldı. Yağışlı sezonda yeteri kadar su bulunduran sığ sulak alanların kurak dönemde suyunu kaybederek tamamen kuruduğu tespit edildi. Yağışlı dönemlerde serbest yüzen yapraklı su bitkileri (özellikle *Azolla filiculoides*, zaman zaman *Salvinia natans* ve *Wolffia arrhiza*) bazı durgun sulak alan yüzeylerini kaplarken, ilkbahar mevsiminde emers otsu bitkilerin (özellikle *Ranunculus sphaerospermus* ve *R. tricophyllus*) sulak alanlarda çok yoğun oldukları tespit edildi. Sulak alanların klimatik faktörlerin yanı sıra antropojenik faaliyetlerden kaynaklı noktasal ve noktasal olmayan çok farklı kirlilik kaynaklarının baskı altında olduğu gözlendi. Ekosistemin işleyişindeki rolü ve önemine dikkat çekmek ve yok olmalarını önlemek için sulak alanlar üzerinde yapılan araştırmalar değerlidir. Faydalı bitki türleri, istenmeyen bitki türlerinin kontrolü, yaban hayatı değerinin en üst düzeye çıkarılması ve diğer fonksiyonları için sulak alanlar iklim değişikliğine uyumlu olarak varlığını devam ettirebilmelidir. Bunun için gerekli önlemler alınmalı, etkili bir sulak yönetim planı ivedi olarak uygulanmalıdır.

Anahtar Kelimeler: Biyolojik çeşitlilik, küresel ısınma, küresel iklim değişikliği, sığ sulak alanlar, su bitkileri

ABSTRACT

Global climate change, which has become evident since the last quarter century, leads to a decrease in fresh water resources, drought and desertification, as well as loss of ecosystem, species and genetic diversity. Climate change is identified as a major threat to wetlands. In recent years, depending on the changing climatic conditions in Turkey, extreme drought has been experienced in the Mediterranean climate region, and sudden and heavy rainfall and flood disasters have been experienced in the Black Sea climate region with drought. In this study, the effect of climate change was observed for four years in some wetlands in Yeşilırmak Delta (Terme, Samsun) located in the Central Black Sea Region. In the rainy (November-March) and dry (June-September) seasons, common aquatic plants that have very

important functions for aquatic ecosystems were identified. It has been determined that shallow wetlands, which have enough water in the rainy season, lose their water in the dry season and dry out completely. While free-floating leafy aquatic plants (especially *Azolla filiculoides*, occasionally *Salvinia natans* and *Wolffia arrhiza*) cover some stagnant wetland surfaces during rainy seasons, emerald herbaceous plants (especially *Ranunculus sphaerospermus* and *R. tricophyllus*) were found to be very dense in wetlands in spring. It has been observed that wetlands are under pressure from many different point and non-point pollution sources due to climatic factors as well as anthropogenic activities. Research on wetlands is valuable in order to draw attention to their role and importance in the functioning of the ecosystem and to prevent their extinction. Wetlands must be able to survive in response to climate change for beneficial plant species, control of undesirable plant species, maximizing wildlife value, and other functions. Necessary measures should be taken for this and an effective wetland management plan should be implemented immediately.
Keywords: Biodiversity, global warming, global climate change, shallow wetlands, aquatic plants

1. GİRİŞ

Günümüzde dünyamızın en büyük sorunlarından birisi küresel iklim değişikliğidir. İklim değişikliği çevreyi ve insan hayatını değişik şekillerde ve olumsuz yönde etkilemektedir. Son çeyrek asırdan itibaren belirgin hale gelen küresel iklim değişikliği su kaynaklarının azalmasına, kuraklık ve çölleşmenin yanı sıra ekosistem, tür ve genetik çeşitlilik kayıplarına yol açmaktadır. Türkiye'de küresel iklim değişikliğiyle ilişkili olarak kuraklık, sel, su kaynaklarının azalması, fırtına ve orman yangınları gibi doğal afetler sıklıkla yaşanmaya başlamıştır. Karasal ve sucul ekosistemlerde meydana gelen bu değişimler nedeniyle ekosistemlerin biyolojik kompozisyonları ciddi risk altındadır. Aynı zamanda sosyoekonomik etkileri de büyüktür.

İklim değişikliği sonucunda, buzulların erimesi, sıcaklık artışı, yağış rejiminde değişiklikler oluşmaktadır (Tourani ve ark., 2022). Amerika Ulusal Havacılık ve Uzay Dairesi (NASA) dünya genelinde yüzey sıcaklığının son 150 yılda arttığını, özellikle son 60 yılda artışın hızlandığını belirtmiştir (NASA, 2022). Geçtiğimiz yirmi yıl içerisinde meydana gelen kuraklık, sıcak hava dalgaları, fırtına ve sel gibi iklimle ilgili afetlerin toplam afetlerin %91'ini oluşturduğu bildirilmiştir (WHO, 2020).

Küresel ısınma ve kuraklığın önemli etkisine bağlı olarak Batı Akdeniz Havzası'nda yer alan Acıgöl, Burdur Gölü ve Eğirdir Gölü son yıllarda önemli oranda sularını kaybetmiştir (Özüpekçe, 2020). Son yıllarda küresel iklim değişikliğinin etkileri Karadeniz Bölgesinde ani seller ve kuraklık olarak hissedilmektedir. Batı Karadeniz'deki Kastamonu ilinde meydana gelen sel felaketi sonucu maddi kayıpların yanı sıra 71 kişi hayatını kaybetmiştir (Çağlayan ve ark., 2021). Orta ve Doğu Karadeniz'de Ordu ve Samsun illerinde 2012, 2016 ve 2019 yıllarında yaşanan sel afeti ciddi maddi hasarlara ve can kaybına yol açmıştır (Taş ve Topaldemir, 2021).

Sucul ekosistemler özellikle sulak alanlar iklim değişikliğinden olumsuz yönde etkilenen hassas alanlardır. Sulak alanlar suya doymuş toprak alanlarıdır ve küçük gölleri, taşkın yataklarını ve bataklıkları içerir (Kayranli ve ark., 2010). Türkiye'de doğal ve/veya insan kaynaklı nedenlerle ekolojik ve hidrolojik olarak olumsuz etkilere maruz kalan doğal göl ve sulak alanların sayısı hızla artmaktadır. Sulak alan ve göllerin nitelik ve niceliklerinin bozulması ağırlıkla iklim değişimine yani klimatolojik faktörlere bağlıdır. Subtropikal bölgede sıcaklığın yükselmesine bağlı olarak buharlaşmanın artması, buna karşın yağışın az düşmesi ile göllerin ve hassas sulak alanların hidrolojik dengesi giderek bozulmaktadır. Doğal göl ve sulak alanlarımızda artan bu tehlike ve oluşacak risklerin önlenmesi amacıyla 2017 yılında "Göller ve Sulak Alanlar Eylem Planı 2017-2023" hazırlanmıştır. Ancak 2017

yılından bu yana göl ve sulak alanlarımızdaki olumsuz etkilerin artarak devam etmesi bu konuda havza ölçeğinde yapılacak olan çalışmaların hızlandırılması ve eksiklerin hızla tamamlanması gereğini ortaya koymaktadır (Yıldız ve ark., 2021).

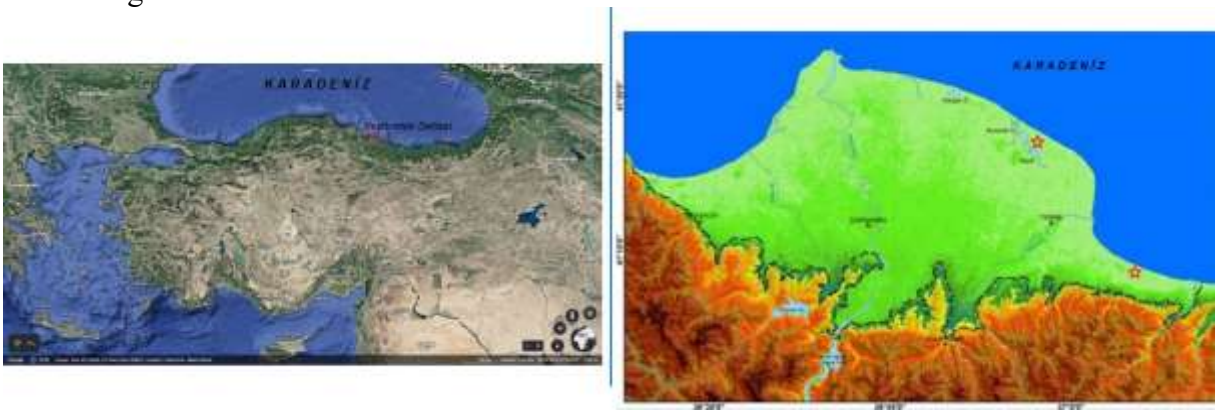
Sulak alanların önemli ekosistem hizmetlerinden biri karbon depolamasıdır. Karbon sekestrasyonu adı verilen karbondioksiti atmosferden uzaklaştırma işleminde sulak alanların çok büyük rolü vardır. Meteorolojik ve hidrolojik koşullara bağlı olarak, sulak alanlar atmosferden karbondioksiti emebilir ve tortu içinde tutabilir. Bu nedenle sulak alanlar sera gazı yutakları olabilir. Sulak alanlarla ilişkili yüksek üretkenlik, yüksek su tablası ve düşük bozunma hızı, toprak, tortu ve döküntü içinde karbon depolanmasına yol açar (Whitting ve Chanton, 2001; Kayranlı ve ark., 2010). Diğer yandan, küresel iklim değişikliğine bağlı olarak değişen hidroloji ve artan sıcaklıklar sulak alanın su kalitesini, biyojeokimyasını ve işlevini değiştirebilir. Örneğin, kuraklık, sulak alanların rolünü karbon yutaklarından karbon kaynaklarına kaydırabilir. Sulak alanlar, özellikle atmosfere metan gazı salımı açısından sera gazı kaynakları olarak kabul edilmektedir. Metan, karbondioksitten çok daha yüksek bir küresel ısınma potansiyeline sahiptir ve kızılötesi radyasyonun atmosferik absorpsiyonuna ve ardından ısınmaya katkıda bulunur (Whitting ve Chanton, 2001; Zhang ve ark., 2005). Bu nedenle, iklim değişikliğiyle mücadelede hayati bir amaç olmalıdır. Sulak alanların korunup iyileştirilmesi yoluyla sera gazlarının salımı azaltılabilir ve aynı zamanda karbon depolamasını artırarak küresel ısınmayı azaltmaya yardımcı olunabilir.

Bu araştırmada, küresel iklim değişikliğinden kaynaklı kuraklığın sulak alanlar üzerindeki etkilerini gözlemlemek amacıyla, Yeşilırmak Deltası'nın doğusunda yer alan (Terme, Samsun) bazı sulak alanlarının durumu yağışlı ve kurak dönemlerde izlenmiştir. Ayrıca sulak alanlarda bulunan ve ekosistemin işleyişinde çok büyük ekolojik önemi olan sucul bitkilerin çeşitliliği de rapor edilmiştir.

2. MATERYAL VE METOT

2.1. ARAŞTIRMA ALANI

Mevcut çalışmada Türkiye'nin kuzeyinde Orta Karadeniz Bölümü'nde bulunan Yeşilırmak Deltasının (Terme, Samsun) doğu bölümünde yer alan sulak alanlar incelendi (Şekil 1). Terme ilçesi topografik özellikleri bakımından aşağı kısımlar ova yukarı bölgeler engebeli ve dağlık alanlardan oluşmaktadır. Denize yakın aşağı kesimlerde 0-10 m yükseltiler ilçenin güneyine doğru gidildikçe artmakta ve yer yer 2000 m yükseltiyi bulmaktadır (Dengiz ve Coşkun, 2016). Araştırma Miliç Kıyı Sulak Alanı (41°12′09.8′′N-37°00′18.6′′E) ile Akgöl Sulak Alanı'nda (41°15′32.6′′N- 36°57′21.0′′E) yapıldı. Akgöl-Simenit kompleksi 1. ve 3. derece doğal sit alanıdır.



Şekil 1. Yeşilırmak Deltası'nın ve çalışma alanlarının lokasyonu

Çalışma alanında her mevsim yağışlı, ılıman geçen Karadeniz iklim tipi görülür (Taş ve Topaldemir, 2021). 2014-2019 yılları arasında Terme-Kozluk meteoroloji istasyonu verilerine göre Terme'nin yıllık ortalama yağış miktarı 100.7 mm, ortalama sıcaklığı ise 14.7 ^{o}C'dir.

Aylık sıcaklık değeri 6.6 °C (Ocak) ila 24.0 °C (Ağustos) arasında değişmektedir (MGM, 2020). Alanda yağışlar Ekim-Mart aylarında, buharlaşma ise Haziran-Eylül ayları arasında maksimum seviyeye ulaşmaktadır.

2.2. ARAZİ İNCELEMELERİ

Arazi çalışmaları 2018-2022 yılları arasında, yağışlı sezonda (ilkbahar ayları) ve kurak sezonda (yaz ve sonbahar mevsimlerinde) yapıldı. Sulak alanlardaki makrofitler toplanıp herbaryum materyali haline getirildi. Alanın fotoğraflanması, sulak alandaki su seviyesi, su rengi, koku gibi özellikleri kayıt altına alındı. Makrofitlerin tanımlanmasında teşhis kitaplarından faydalanıldı (Altınayar, 1988; Seçmen ve Leblebici, 2008; DSİ, 2009; Güner ve ark., 2018). Sucul bitkilerin vejetatif ve generatif organlarının bulunmasına ve gelişimlerinin olgun dönemlerinde toplanmasına özen gösterildi (Özbucak ve Taş, 2016).

3. BULGULAR VE ANALİZ

Yeşilırmak Deltası'nda lagünler, bataklıklar, sulak alanlar, su basar ormanlar, plajlar, koruma altına alınmış doğal yaşam alanları gibi çok çeşitli ekosistem sahaları mevcuttur. Oldukça düz bir topografyaya sahip olmasına rağmen deltadaki birçok sulak alana ulaşım oldukça zordur. Kaynağını deltanın güneyindeki dağlık araziden alan çok sayıda akarsu delta üzerinden akarak Karadeniz'e dökülmektedir. Taban suyu seviyesinin yüksek olmasına bağlı olarak yılın büyük bir bölümünde özellikle yağışlı sezonlarda bataklıklar oluşabilmektedir. Yine deltada eğimin düşük olmasına bağlı olarak drenaj problemleri yaşanmaktadır. Bu nedenle kıyısal ekosistemlere araçla ulaşmak sıkıntılıdır.

Türkiye'nin önemli tarım arazilerine sahip olan Yeşilırmak Deltası'nda doğal alanların çoğu tarım alanlarına dönüştürülmüştür. Burada fındık, mısır, pirinç, kavak yetiştiriciliği yaygın olarak yapılmaktadır. Dolayısıyla deltadaki sulak alanlar insan kaynaklı tarımsal faaliyetlerden birinci derecede etkilenmektedir. Delta, özellikle sel ve taşkınlara bağlı olarak doğal afetlerin de ciddi baskısı altındadır.

İnsan kaynaklı noktasal ve noktasal olmayan çok farklı kirlilik kaynaklarından sulak alanlar baskı altındadır. Kıyısal ekosistemde Miliç sulak alanında konumlanmış, yine Akgöl ve çevresindeki dağınık yerleşim alanlarında evsel atıklar/atıksular, foseptik sızıntılar, hayvan yetiştiriciliği ve ahırlardan sızıntı sular ve gübreler, tarım arazilerinde kimyasal gübreleme ve pestisit uygulamaları ve bu alanlardan gelen drenaj kanalları sulak su kalitesi ve trofik durumunu etkileyen en önemli faktörlerdir. Bunun yanı sıra, Karadeniz sahil karayolundan kaynaklı kirlilik, düzensiz ve aşırı hayvan otlatma, saz kesimi, aşırı balık avcılığı gibi faktörler de sulak alan ekosistem işleyişini etkileyen faktörlerdendir.

İnsan kaynaklı faktörlerle birlikte özellikle son yıllarda bölgede ciddi etkilerini gösteren küresel iklim değişikliği sulak alanlar üzerinde önemli bir baskı unsurudur. Klimatik faktörlere bağlı olarak alanda sel ve kuraklık olayları sürekli/geçici sulak alanların işleyişini oldukça etkilemektedir. Yaz sezonunda sulak alanlardaki ve lagünlerdeki su seviyesinin azaldığı, geçici sulak alanlar ve bataklıkların kuruduğu, suların humik renge dönüştüğü, bulanıklığın oldukça arttığı, oksijen yetersizliğine bağlı olarak balıkların su yüzeyinde toplandığı, istilacı balıkların ve istilacı su bitkilerinin aşırı çoğaldığı dikkat çeken durumlardır. Sucul ekosistemlerde gözlenen bu durumlar ekosistem, tür ve gen çeşitliliğini tehdit eden önemli hususlardır.

Akgöl sulak alanında yağışlı sezonda su derinliği 30-40 cm'ye kadar ulaşabilmektedir. Sıcaklığın yükselmesi ve kuraklığın etkisiyle yaz mevsiminde göl su seviyesi azalırken özellikle geçici sulak alanların sularının tamamen kuruduğu gözlenmiştir (Şekil 2). Yağışlı dönemlerde serbest yüzen yapraklı su bitkileri (*Azolla filiculoides* ve *Wolffia arrhiza* türleri) su yüzeyini yer yer kaplamaktadır. Yine ilkbahar mevsiminde emers bitkilerden *Ranunculus sphaerospermus* ve *R. tricophyllus* türlerinin sulak alanlarda çok yoğun oldukları tespit edilmiştir (Şekil 2). Benzer ekolojik durumlar Miliç kıyı sulak alanı için de gözlemlenmiştir, ancak Miliç sulak alanının su seviyesi Akgöl sulak alanına göre daha yüksektir.

Şekil 2. Akgöl ve Miliç çevresinde geçici sulak alanların yağışlı ve kurak sezondaki durumu.

Geçici sulak alanlarda yaygın ve bol olarak bulunan on iki farklı sucul bitki türü belirlendi: *Alisma plantago-aquatica* L., *Azolla filiculoides* Lam., *Callitriche stagnalis* Scop., *Cynodon dactylon* (L.) Pers., *Juncus acutus* L., *Lemna minor L., Polygonum* sp., *Ranunculus repens* L., *R. sphaerospermus* Boiss. & Balanche, *R. trichophyllus Chaix*, *Wolffia arrhiza* (L.) Horkel ex Wimm., *Zannichellia palustris* L. (Tablo 1, Şekil 3). Bu türler tatlı suya sahip sığ sularda, göl ve nehir kıyılarında, sulama kanallarında, bataklıklar veya nemli topraklarda özellikle yağışlı sezonda yaygın olarak gözlendi (Şekil 3).

Şekil 3. Geçici sulak alanlarda yaygın bulunan sucul bitkiler

Tablo 1. Sulak alan ve bataklıklarda yaygın bulunan makrofitler

Kingdom	Phylum	Class	Order	Family	Genus	Species
Plantae	Tracheophyta	Polypodiopsida	Salviniales	Salviniaceae	*Azolla*	*A. filiculoides* Lam.
Plantae	Tracheophyta	Liliopsida	Alismatales	Araceae	*Lemna*	*L. minor* L.
Plantae	Tracheophyta	Liliopsida	Alismatales	Araceae	*Wolffia*	*W. arrhiza* (L.) Horkel ex Wimm.
Plantae	Tracheophyta	Liliopsida	Alismatales	Potamogetonaceae	*Zannichellia*	*Z. palustris* L.
Plantae	Tracheophyta	Liliopsida	Poales	Juncaceae	*Juncus*	*J. acutus* L.
Plantae	Tracheophyta	Liliopsida	Poales	Poaceae	*Cynodon*	*C. dactylon* (L.) Pers.
Plantae	Tracheophyta	Magnoliopsida	Alismatales	Alismataceae	*Alisma*	*A. plantago-aquatica* L.
Plantae	Tracheophyta	Magnoliopsida	Caryophyllales	Polygonaceae	*Polygonum*	*Polygonum* sp.
Plantae	Tracheophyta	Magnoliopsida	Lamiales	Plantaginaceae	*Callitriche*	*C. stagnalis* Scop.
Plantae	Tracheophyta	Magnoliopsida	Ranunculales	Ranunculaceae	*Ranunculus*	*R. repens* L.
Plantae	Tracheophyta	Magnoliopsida	Ranunculales	Ranunculaceae	*Ranunculus*	*R. sphaerospermus* Boiss. & Blanche
Plantae	Tracheophyta	Magnoliopsida	Ranunculales	Ranunculaceae	*Ranunculus*	*R. trichophyllus* Chaix

4. TARTIŞMA VE SONUÇ

Yeşilırmak Deltası'ndaki Akgöl ve Miliç sulak alanlarında yapılan incelemelerde, iklim değişikliği ve küresel ısınmanın sulak alan ekosistemleri üzerinde olumsuz etkiler yaptığı gözlenmiştir. Yağışlı dönemde yeterince su bulunduran sulak alanların, kurak sezonda yağışların azalması ve aşırı buharlaşma nedeniyle sularının azaldığı, geçici sulak alanların ise tamamen sularını kaybederek kuruduğu tespit edilmiştir. Yeşilırmak alt havzası olan Çarşamba Ovası'nda yoğun tarımsal faaliyetler yapılmaktadır. Yerüstü ve yeraltı sularının tarımsal sulamada kullanılması da sulak alanlardaki su miktarının azalmasında etkilidir. Zaman zaman alanda yağışlı sezonlarda yaşanan sel sonucu da sulak alan ekosistemini olumsuz etkilenmekte, yukarı havzadan özellikle tarım ve yerleşim alanlarından taşınan noktasal/yayılı kirlilik yükü aşağı havzadaki sulak alanlarda birikmekte, suda organik/inorganik yük ve bulanıklık artmaktadır. Böyle durumlarda sucul flora ve fauna olumsuz etkilenmektedir.

Küresel iklim değişikliği ile oluşan sıcaklık artışı ve kuraklığın sucul ekosistemler üzerindeki etkilerini konu alan araştırmalar oldukça önemlidir. Karadeniz kıyısındaki diğer önemli bir sulak alan ekosistemine sahip Kızılırmak Deltası'nda yapılan bir çalışmada, deltanın kuraklık durumu değerlendirilmiştir. Araştırma kapsamında, Bafra meteoroloji istasyonu verilerine göre, son 53 yılın 25 yılının yağışlı, 28 yılının kurak olduğu; Samsun meteoroloji istasyonu verilerine göre ise son 87 yılın 39 yılının yağışlı, 48 yılının ise kurak geçtiği bildirilmiştir. Çalışmada, 2000 yılından sonra kurak dönemlerin baskın olduğu belirtilmiştir (Şener, 2021). Yine, Akdeniz iklim kuşağında, Batı Akdeniz Havzası'ndaki Burdur Gölü, Acıgöl ve Eğirdir Gölü'nde kuraklaşan iklim koşullarından dolayı önemli su kayıplarının olduğu belirtilmiştir (Özüpekçe, 2020). Manavgat Çayı'nda kuraklık indisi (SDI) kullanılarak hidrolojik kuraklık durumunun belirlendiği çalışmada, akarsuyun değişik dönemlerde hidrolojik kuraklık yaşadığı tespit edilmiştir (Kale, 2021). Uluslararası çalışmalarda da küresel iklim değişikliği ve kuraklığın etkilerinin belirlenmeye çalışıldığı bir araştırmada, İran'ın önemli göllerinden olan Urmiye Gölü'nün son 20 yılda kuraklık ve küresel iklim değişikliği sonucu göl yüzey alanının 5500 km^2'den 1661 km^2'ye düştüğü tespit edilmiştir (Mohebbi ve ark., 2020).

Yapılan araştırmalar, klimatik faktörlerin özellikle hidrolojik kuraklığın sucul ekosistem biyoçeşitliliği üzerinde önemli etkileri olduğu vurgulamaktadır. Çalışma alanımızda da iklim değişikliğine bağlı yaşanabilecek uzun süreli kuraklık makrofit biyoçeşitliliğini olumsuz yönde etkileyebilir. Bu durumda sucul ekosistemler sistem için anahtar rol oynayan sucul bitkilerin ekolojik etkinliklerinden yoksun kalabilir. Çünkü sulak alanlarda farklı türlerden yayılış gösteren makrofitler, içerisinde bulunduğu suyun fiziksel ve kimyasal özellikleri

üzerinde doğrudan/dolaylı etkilidir. Sucul bitkiler sulak alandaki özellikle sel zamanı artan suyun bulanıklığını azaltırken, azot ve fosfor gibi nütrientleri filtre ederek sudan uzaklaştırır, fotosentez yaparak suda çözünmüş oksijen konsantrasyonunu artırırlar. Submers bitkiler sedimentin sabitlenmesini sağlayıp, sedimentteki nütrientlerin suya salımını azaltırlar (Taş ve Topaldemir, 2021). Sularda kirlenmeye sebep olan iz elementleri bünyelerine alarak suyun temizlenmesine yardımcı olurlar (Yılmaz ve Taş, 2021). *Elodea* ve *Chara* gibi bazı sucul bitkiler su sertliğinin azalmasına katkı sağlarlar (Cirik ve ark., 2000). Sulak alan ekosistemleri için oldukça önemli fonksiyonları olan makrofitlerin peyzaj çalışmalarında da kullanım potansiyeli bulunmaktadır (Taş ve ark., 2018).

Küresel iklim değişikliği sucul ekosistemin ekolojik koşullarını değiştirirken, değişen ortam şartlarına uyum gösteren sucul istilacı türler alanda hızla çoğalmakta ve yayılış alanını genişletmektedir (Taş ve ark., 2019). Çalışma alanımızda serbest yüzen yapraklı makrofitlerden *Azolla filiculoides*, *Salvinia natans* ve *Wolffia arrhiza* türlerinin yer yer sulak alan ve bataklıklarda aşırı çoğaldığı gözlenmiştir (Şekil 4). Sulak alanların litoral bölgeleri de yoğun bir *Phragmites australis* toplulukları ile çevrilidir. Sucul ekosistemlerde istilacı bir balık türü olan İsrail sazanının hızla çoğalması da dikkat çekmektedir (Şekil 5).

Şekil 4. Serbest yüzen yapraklı su bitkilerinin (*Azolla filiculoides*, *Salvinia natans*, *Wolffia arrhiza*) su yüzeyini kaplaması

Şekil 5. Küresel iklim değişikliğinin etkilerinden biri, sel afeti ve bulanık suda balıklar

Su yüzeyinin yoğun bir şekilde su bitkisi ile kaplı olması maksimum karbon tutumu sağlayabilir. Xiaonan ve ark. (2008), bataklıkların karbon tutma potansiyelinin göllerinkinden çok daha yüksek olduğunu bildirmiştir. Ancak, kurak sezon boyunca devam eden istilacı türlerin yoğunluğu, sulak alan ekosistemindeki diğer türler için gölgeleme, su ve atmosfer

arasında ışık ve gaz geçirgenliğinin engellenmesi, sulak alan yüzeyinin daralması, sedimentte organik madde birikiminin artması, su seviyesinin azalması, dekompozisyon olayının artması neticesinde anoksik koşulların meydana gelmesi ve sucul flora ve faunanın bu durumdan olumsuz etkilenmesi gibi sonuçlara yol açmaktadır. Turcq ve ark. (2002) kuru iklimlerde düşük karbon depolanırken nemli iklim bölgelerindeki çoğu gölde yüksek karbon birikiminin gerçekleştiğini belirtmiştir. Çalışma alanımızın her mevsim yağışlı, şimdilik nemli bir iklime sahip olması bir avantaj olarak değerlendirilebilir.

Brix et al. (2001), *P. australis*'in hâkim olduğu sulak alanlarda birincil üretkenliğin yüksek olduğunu ve net birincil üretimin yaklaşık %50'sinin tortudaki karbondioksit ve metan aracılığıyla solunduğunu bildirmiştir. Dolayısıyla, çalışma alanımızdaki yoğun *P. australis* toplulukları hem sulak alan işleyişinde hem de küresel ısınmanın azaltılmasında önemli rol oynamaktadır. Euliss ve ark. (2006), sulak alanların, işlenmemiş ekili araziye kıyasla organik karbonun yaklaşık iki katı kadar depolayabildiğini ifade etmiştir. Landry ve ark. (2009), bitkili sulak alanların karbondioksit olarak yaydıklarından 2 ila 15 kat daha fazla karbon tutabileceğini bildirmiştir.

Yoğun zirai faaliyetlerin yapıldığı çalışma alanımızda, sulak alanlar üzerinde beşerî faktörlerin de etkileri gözlenmiştir. Sulak alan bitkilerinin düzensiz kesilmesi, yakılması, kanal temizleme faaliyetleriyle yoğun organik materyal içeren sedimentin açığa çıkarılması, evsel atık/atıksuların sulak alanlara bırakılması, foseptik ve ahır suları ile tarım alanlarından gelen drenaj kanallarının sulak alanlara bağlanması, bazı bataklık alanların doldurulması vb. Sulak alanların tarıma açılmasının önüne geçilmeli ve mevcut tarım arazileri iyileştirilerek tarımsal faaliyetler yapılmalıdır (Korkanç ve ark., 2022). Türkiye'de en fazla su tarımsal faaliyetlerde kullanılmaktadır. Mevcut su kaynaklarını korunması için su yönetiminin önemi büyüktür. Akıllı tarım uygulamaları ve uygun sulama yöntemleri ile su tasarrufu sağlanabilir, böylece sulak alanlardan çok fazla su çekilmez.

Küresel ısınmanın etkilerini azaltmak için sera gazı salımlarının sınırlandırılması veya azaltılması önemli çalışmalardır. Sulak alanlarda karbon depolamasına ilişkin çalışmalar sulak alanların hayati karbon yutakları olduğunu göstermektedir (Kayranlı ve ark., 2010). İklim değişikliği tüm dünyayı etkilemekte ve etkileri de uzun sürmektedir. Dolayısıyla yeryüzünde yaşamın iklim değişikliğinin etkilerine hazırlıklı olmalı ve bu etkinin ortadan kaldırılması için çaba göstermelidir (Türkeş, 2021). İnsanlar geçim kaynaklarını, sağlığını, gıda ihtiyacını, biyoçeşitliliği ve ekosistem bütünlüğünü korumak için küresel iklim değişikliğine direngen ve uyumlu hale getirilmelidir (Türkeş, 2022). İklim değişikliğine uyum ve risk azaltma politikaları (Kazancı ve Tezer, 2021) ile kentsel büyüme ve arazi kullanım politikaları geliştirilmelidir (Ren ve ark., 2020; Altınok, 2022).

Küresel iklim değişikliğinin etkileri azaltıldığında sulak alanların ekolojik yapıları korunacaktır. Karbonu bünyesinde depolayabilme özelliğine sahip sulak alanların sürdürülebilirliğini sağlamak ekosistem sürdürebilirliği açısından çok önemli bir konudur. Ancak, sıcaklık artışları devam ederse ve daha hızlı hale gelirse, organik maddenin ayrışmasının artacağı ve sulak alanların daha sonra ana karbon kaynakları haline geleceği unutulmamalıdır. Çünkü, tatlı su sulak alanları, atmosferik karbon için potansiyel bir yutak sağlar, ancak uygun şekilde yönetilmezse, karbondioksit ve metan gibi sera gazı kaynakları haline gelebilir.

KAYNAKÇA

Altınayar, G. (1988). Su yabancı otları. Bayındırlık ve İskân Bakanlığı Devlet Su İşleri Genel Müdürlüğü İşletme ve Bakım Dairesi Başkanlığı Yayınları, Ankara.

Altınok, G. K. (2022). Kentsel Büyüme Dinamiklerinin İklim Değişikliği Etkileri Çerçevesinde Yeniden İrdelenmesi. *Çevre Şehir ve İklim Dergisi,* 1(1): 174-198.

Brix, H., Sorrell, B.K., & Lorenzen, B. (2001). Are *Phragmites*-dominated wetlands a net source or net sink of greenhouse gases. *Aquatic Botany*, 69: 313–324.

Cirik, S., Cirik, Ş., & Conk-Dalay, M. (2000). Su Bitkileri II (İçsu Bitkilerinin Biyolojisi, Ekolojisi, Yetiştirme Teknikleri). Ege Üniversitesi Su Ürünleri Fakültesi Yayınları No: 61, Ders Kitabı Dizini No: 28, Bornova, İzmir.

Çağlayan, A., Saber, R., & Işık, V. (2021, October 10-14). Assessment of August 11, 2021 Bozkurt flood disaster with Sentinel-2 satellite images, Turkey. [Conference presentation]. 11th Congress of the Balkan Geophysical Society, Bucharest.

Dengiz, O., & Coşkun, A. (2016). Samsun Terme Havzası bazı temel fizyografik karakteristikleri belirlenmesi ve tarımsal taşkın alanlarının toprak haritalanması. *Türkiye Tarımsal Araştırmalar Dergisi*, 3(1): 1−13.

DSİ, (2009). Su Yabancıotları, Yayılış Alanları, Yaşamları, Çevresel İlişkileri, Sorunları ve Savaşım Yöntemleri. T.C. Çevre ve Orman Bakanlığı, Devlet Su İşleri Genel Müdürlüğü, İşletme ve Bakım Dairesi Başkanlığı, DSİ İdari ve Mali İşler Dairesi Başkanlığı Basım ve Foto-Film Şube Müdürlüğü, Ankara.

Euliss, N.H. Jr, Gleason, R.A., Olness, A., McDougal, R.L., Murkin, H.R., Robarts, R.D., Bourbonniere, R.A., & Warner, B.G. (2006). North American prairie wetlands are important nonforested land-based carbon storage sites. *Science of the Total Environment*, 361: 179–188.

Güner, A., Aslan, S., Ekim, T., Vural, M., & Babaç, M.T. (Eds.) (2018). Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi Yayınları Flora Dizisi 1, İstanbul.

Kale, M.M. (2021). Manavgat Çayı Kuraklık Analizi. *Ege Coğrafya Dergisi*, 30(1): 107–123.

Kayranli, B., Scholz, M., Mustafa, A., & Hedmark, Å. (2010). Carbon storage and fluxes within freshwater wetlands: a critical review. *Wetlands*, 30(1): 111−124.

Kazancı, G., & Tezer, A. (2021). "İklim Değişikliğine Uyumda Mekânsal Planlama ve Akıllı Yönetişim Çerçevesinde Türkiye". *Planlama Dergisi*, 31(2): 302−320.

Korkanç, S. Y., Korkanç, M., Mert, M. H., Geçili, A., & Serengil, Y. (2022). Arazi kullanımının sulak alandan tarıma dönüştürülmesinin bazı toprak özellikleri üzerindeki etkileri ve bunların toprak derinliği ile değişimi. *Niğde Ömer Halisdemir Üniversitesi Mühendislik Bilimleri Dergisi*, 11(2): 246−257.

Landry, G.M., Maranger, R., Brisson, J., & Chazarenc, F. (2009). Greenhouse gas production and efficiency of planted and artificially aerated constructed wetlands. *Environmental Pollution*, 157: 748–754.

MGM, (2020). Meteoroloji Genel Müdürlüğü (MGM), Meteoroloji 10. Bölge Müdürlüğü, Samsun.

Mohebbi, F., Dadash pour, B., & Mohebbi Rad, H. (2020). Exploring the causes and consequence of Lake Urmia environmental disaster. *Ecology of Water Resources Journal*, 4(1): 49-61. https:// ewrj.areeo.ac.ir/article_122379.html?lang=en

NASA. (2022). National Aeronautics and Space Administration Goddard, GISS Surface Temperature Analysis (GISTEMP v4). Retrieved from https://data.giss.nasa.gov/gistemp/

Özbucak, T. & Taş, B. (2016). Kacalı Deresi riparian alanının (Perşembe, Ordu) makrofit florası. *Akademik Ziraat Dergisi*, 5(2): 117−125.

Özüpekçe, S. (2020). Batı Akdeniz Havzaları ve yakın çevresinde kuraklık eğilimi ve su kaynakları ile ilişkisi. *lnternational Journal of Geography and Geography Education*, (43): 317−337.

Ren, J., Zhou, W., Liu, X., Zhou, L., Guo, J., Wang, Y., & Ma, R. (2020). Urban expansion and growth boundaries in an oasis city in an arid region: A case study of Jiayuguan City, China. *Sustainability*, 12(1): 210. https://doi.org/10.3390/su12010210

Seçmen, O., & Leblebici, E. (2008). Türkiye sulak alan bitkileri ve bitki örtüsü. Ege Üniversitesi Fen Fakültesi Yayınları No: 158, Bornova, İzmir.

Şener, E. (2021). Standartlaştırılmış Yağış İndeksi (SYİ) Yöntemi ile Kızılırmak Deltası (Samsun) Kuraklık Analizi. *Academic Perspective Procedia*, 4(2): 99−105.

Taş, B., & Topaldemir, H. (2021). Assessment of aquatic plants in the Miliç Coastal Wetland (Terme, Samsun, Turkey). *Review of Hydrobiology*, 14(1−2): 1−23.

Taş, B., Ertürk, Ö., Yonat, H., & Topaldemir, H. (2019). Son yıllarda Yeşilırmak Deltası'ndaki sulak alanlarda istilacı bir su bitkisi: "yeşil altın madeni" kızıl eğrelti (*Azolla filiculoides* Lam.). 3. Uluslararası ÜNİDOKAP Karadeniz Sempozyumu, 21-23 Haziran 2019, Tokat, Sempozyum Kitabı, 424−436.

Taş, Y., Taş, B., & Topaldemir, H. (2018). Yeşilırmak Deltası'ndaki (Samsun) sulak alanlarda yaygın bulunan su bitkilerinin peyzaj mimarlığında kullanım potansiyelleri, 1st International Congress on Plant Biology, 10–12 May, Konya.

Tourani, M., Çağlayan, A., Işık, V., & Saber, R. (2022). İran'da iklim değişikliğinin, klimatolojik, meteorolojik ve hidrolojik afetlere etkisi. *Türk Coğrafya Dergisi*, (80): 97−114.

Turcq, B., Cordeiro, R.C., Albuquerque, A.L.S., Sifeddine, A., Simoes Filho, F.F.L., Souza, A.G., Abrao, J.J., Oliveira, F.B.L., Silva, A.O., & Capitaneo, J.A. (2002). Accumulation of organic carbon in five Brazilian lakes during the Holocene. *Sedimentary Geology*, 148: 319–342.

Türkeş, M. (2021). Toplumun iklim değişikliği direngenliği güçlendirilebilir mi? Spektrum, Kasım, 6, 95−101.

Türkeş, M. (2022). IPCC'nin Yeni Yayımlanan İklim Değişikliğinin Etkileri, Uyum ve Etkilenebilirlik Raporu Bize Neler Söylüyor?. *Resilience*, 6(1): 197−207.

Whiting, G. J., & Chanton, J.P. (2001). Greenhouse carbon balance of wetlands: methane emission versus carbon sequestration. *Tellus B*, *53*(5): 521−528.

World Health Organization, (2020). Coronavirus disease 2019 (COVID- 19). situation report, 51. World Health Organization. https://apps.who.int/iris/handle/10665/331475

Xiaonan, D., Xiaoke, W., Lu, F., & Zhiyun, O. (2008). Primary evaluation of carbon sequestration potential of wetlands in China. *Acta Ecologica Sinica* 28: 463–469.

Yıldız, D., Özgüler, H., & Kandemir A.F. (2021). KÖMÜR-ENERJİ-SU. Kömür yakıtlı termik santrallerimizin su kullanımları ve atıkları. Su Politikaları Derneği, Rapor No: 40, Ankara.

Yılmaz, Ö., & Taş, B. (2021). Feasibility and assessment of the phytoremediation potential of green microalga and duckweed for zeta-cypermethrin removal. *Desalination and Water Treatment*, 209: 131−143.

Zhang, J.B., Song, C.C., & Yang, W.Y. (2005). Cold season CH_4, CO_2 and N_2O fluxes from freshwater marshes in northeast China. *Chemosphere*, 59(11): 1703−1705.

INVESTIGATION OF BIOINDICATOR POTENTIAL OF COMMON CARP FOR THE MONITORING OF MICROPLASTIC POLLUTION IN FRESHWATER ENVIRONMENTS

Dr. Ece KILIÇ
Iskenderun Technical University, Faculty of Marine Science and Technology, Hatay/Turkey

ABSTRACT
Aquatic environments are suffer from extensive microplastic (MP) contamination. Even though microplastic pollution in marine environments has been extensively studied, information regarding the MP contamination levels in freshwater environments is very limited. Since the biophysicochemical influences in freshwater environments were completely different from marine environments, fish prone to microplastic pollution may influence more drastically than marine fish. For that reason, this study was designed to evaluate the biomonitoring potential of common carp *Cyprinus carpio* Linnaeus, 1758 in freshwater environments. In this context, microplastic ingestion rate and mean MPs abundance were obtained from the literature. Available knowledge regarding its benthopelagic habitat, widespread stock, tolerance to contaminants, global catchment and production rate makes this species a perfect candidate for MPs monitoring. In addition, high microplastic detection frequency, even 100% in some cases, and high MPs abundance in the gastrointestinal tract reported in the previous literature showed that this specie can be used to monitor MPs contamination as a sentinel species. Besides, even though the danger of fish consumption due to MPs contamination is debatable, previous literature showed that necessary national and international measures needs to be taken.
Keywords: *Cyprinus carpio*, microplastic ingestion, plastic litter

1. INTRODUCTION
Plastics which are less than 5 mm in size were called as microplastic particles, and they have become an important pollutant in aquatic environments. As a consequence of the global increase in plastic usage, their abundance in aquatic environments has been increasing sharply. This condition increases the encounter risk of aquatic animals with MPs which potential impacts of these emerging contaminants on aquatic animals have become a major concern.

In literature, microplastic pollution in marine environments has been extensively studied; however, information in freshwater environments is very limited. Also, marine and freshwater environments were completely different in terms of hydrological and biological properties, and freshwater environments were generally more susceptible to microplastic pollution and contain a completely different set of chemicals (Scherer et al., 2018). For that reason, the interaction between MPs and freshwater biota carries significant importance.

In this study, bioindicator potential of common carp was evaluated by examining available data from the literature. Common carp is selected as a candidate, because it is (i) a globally distributed species and have dominant stock from Europe to Asia (Froese and Pauly, 2022), (ii) benthopelagic species and fed from both the benthic environment and water column which allow us to evaluate MPs existence in different environments, (iii) tolerant to diverse environments (Froese and Pauly, 2022) and resistant to pollutants (Lee et al., 2012).

2. DATA COLLECTION AND EVALUATION
To obtain data from the literature, scientific databases like Google Scholar, Web of Science, and Scopus were used. Keyword combinations were selected as “microplastic ingestion AND

fish", "microplastic ingestion AND common carp", and "microplastic ingestion AND *Cyprinus carpio*". Documents were extracted till August 18, 2022, and data from 11 studies (including article and thesis) were obtained from the literature.

Information regarding the number of specimens, mean number of ingested MPs, standard error (if available), and frequency of MPs occurrence were extracted from the literature (Table 1). For the studies in which MPs occurrence in the GIT is less than 100%, the minimum number of ingested MPs was taken as 0. For others, the minimum and maximum of ingested MPs were calculated by extracting/subtracting the mean number of ingested MPs and standard error, respectively.

3. MICROPLASTIC ABUNDANCE IN COMMON CARP

Microplastic ingestion rate of common carp was varied depending on the study site. In increasing order, microplastic occurrence in the gastrointestinal tract of common carp was reported as 15% in Karasu River, Turkey (Atamanalp et al., 2022), 16% in Pearl River, China (Zheng et al., 2019), 31% in Thames River, Ontario (Wardlaw, 2021), 35% in Lake Ziway, Ethiopia (Merga et al., 2020), 40-50% in Sürgü Dam Reservoir, Turkey (Turhan, 2022) and 100% in Han River, South Korea (Park et al. 2020), Vaal River, South Africa (Saad et al., 2022), Orontes River, Turkey (Kılıç et al., 2022).

Similar to microplastic ingestion rates, number of MPs extracted from the GIT of common carp were different depending on studies. The highest abundance was reported from Han River, South Korea (Park et al. 2020) and Vaal River, South Africa (Saad et al., 2022) which were followed by Thames River, Ontario (Wardlaw, 2021) and Karasu River, Turkey (Atamanalp et al., 2022). On the other hand, the lowest mean abundance was reported from Sürgü Dam Reservoir, Turkey (Turhan, 2022) (Table 1).

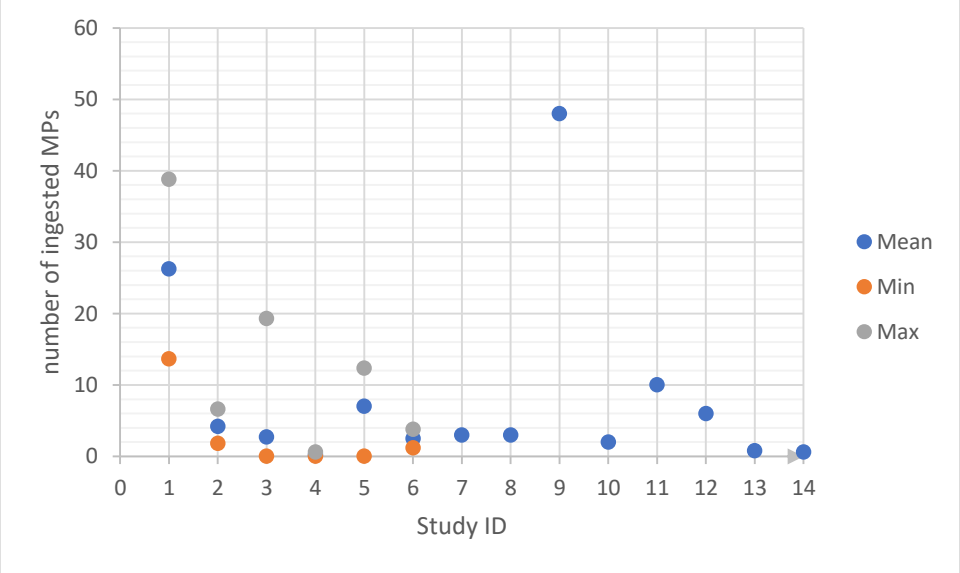


Figure 1. Minimum, maximum and mean number of ingested MPs from the GIT of common carp. (In the x scale, study ID refers to the ıdentification code defined in Table 1).

Figure 1 represents variations in mean MPs abundance in the GIT of common carp from the literature. It showed that MPs amount in the GIT varies in regional scale. Also, number of MPs extracted from a single specimen were varied within and between the studies. Therefore, Figure shows that, in addition to regional differences, variations in the behavior of studies specimens like hunger satiety, sampling time alter the MPs ingestion rates.

Previous studies showed that as the MPs contamination in the freshwater system increases, MPs ingestion of freshwater fish increases (Wardlaw, 2021). Therefore, it is thought that most important factor impacting the MPs uptake of fish was MPs contamination level in the ambient environment. In this sense, differences observed in the previous literature is mainly

arise from the differences in the anthropogenic activities taking place in the surrounding environment. In addition, methodological differences, hydrodynamic circulation and/or major anthropogenic facilities employed the watershed cause variations in the MPs ingestion rates (Pellini et al., 2018; Zhang et al., 2020)

Despite from the differences observed between studies, the presence of MPs in every conducted study is a proof that common carp is strongly influenced by the MPs presence in the freshwater environments. This condition may pose a threat the health and well-being of common carp. Previous studies reported oxidative stress, inhibition in weight loss and growth, psychical changes such as abnormal swimming patterns, impaired movement coordination, and alterations in biochemical balance of fish like changes in liver enzymes, kidney functions (Chen et al., 2022; Hamed et al., 2022). Therefore, MPs contamination in freshwater environments is a significant issue for health of environments and important problem for fish farms considering the intense aquaculture potential of common carp.

4. EVALUATION OF BIOINDICATOR POTENTIAL OF COMMON CARP

As described above, MPs contamination is a global problem to sustain the quality of freshwater environments as well as the sustainable growth of the aquaculture industry. In this manner, the selection of suitable bioindicator species is a crucial step to determine the hazard potential of MPs contamination.

Fossi et al. (2018) identified the selection criteria to access the suitability of species as bioindicators. They identified that a bioindicator species should have enough information regarding its ecology, biology, habitat, feeding habit, and trophic level. Since common carp is a well-known species since 1980, literature reporting their bio-ecology is comprehensive enough to use this specie as a bioindicator.

In addition, common carp is a widely distributed species; therefore, comparison between different regions is possible. In addition, these species are tolerant to poor environmental conditions (Lee et al., 2012), and can be used as a bioindicator in areas where other fish species could not survive.

Besides, common carp is a benthopelagic species and fed from both the benthic environment and the water column. Considering its high microplastic ingestion rate reported by the previous studies, this species could be used to determine potential sources of MPs from different habitats.

Finally, the common carp is a popular human food source. Therefore, they can also be used to monitor the potential transfer of MPs to humans through trophic transfer.

5. IMPORTANCE OF COMMON CARP FOR FISHERIES

Common carp is an important food source and has significant commercial value. The global catchment rate of common carp has increased by 28-fold since 1980, and the highest catching rate belongs to Turkey and Thailand (FAO, 2022).

As a parallel to the high demand for common carp for popular seafood, carp farming has become a significant industry.

Global aquaculture production of common carp was more than 4 million tones in 2020 (FAO, 2022)

Table 1. Microplastic ingestion rates by common carp worldwide

Study Number	Site	n	Extraction Method	Mean MPs Abundance (particles/fish)	FO (%)	Color	Type	Reference
1	Vaal river, South Africa	26	10% KOH	26.23 ± 12.57	100	Green	fiber	Saad et al., 2022
2	Orontes River, Turkey	19	30% H_2O_2	4.2±2.4	100	Black	fiber	Kılıç et al., 2022
3	Thames River, Ontario	56	20% KOH	2.69±16.62	31	Black	fiber	Wardlaw, 2021
4	Pearl River, China	19	30% H_2O_2	0.2±0.4	15,8	Transparent	fiber	Zheng et al., 2019
5	Karasu River, Turkey	25	30% H_2O_2	7±5.34	15	Black	fiber	Atamnalp et al., 2022
6	China	20-40	30% H_2O_2	2.5±1.3		Transparent	fiber	Jabeen et al., 2017
7	Lake Ziway, Ethiopia	30	10% KOH	3	35	Transparent and blue	fragment	Merga et al., 2020
8	Lake Ziway, Ethiopia	30	10% KOH	3	35	Transparent and blue	fragment	Merga et al., 2020
9	Han River, South Korea	1	10% KOH	48	100		fragment	Park et al., 2020
10	Anzali wetland, Iran	31	10% KOH	2	-	Black	fiber	Rasta et al., 2021
11	Qarasu River	13	30% H_2O_2	10	-	Black	fiber	Hedayati, 2022
12	Lake Ziway, Ethiopia	30	10% KOH	6	35	Transparent and blue	fragment	Merga et al., 2020
13	Sürgü Dam Reservoir, Turkey	20	30% H_2O_2	0.8	50	Transparent	Fiber	Turhan, 2021
14	Sürgü Dam Reservoir, Turkey	20	30% H_2O_2	0.6	40	Transparent	Fiber	Turhan, 2021

6. CONCLUSION

Overall, literature regarding the microplastic ingestion rate of common carp is heterogeneous. Therefore, variations in the microplastic ingestion rates could be used to evaluate temporal variations differences in representing the MPs contamination levels in different environments when a similar methodology was followed. In addition, considering benthopelagic habitat, widespread stock, tolerance to contaminants and global catchment and production rate, common carp can be used as bioindicator species for the monitoring of MPs contamination in freshwater environments. Also, considering the widespread usage of common carp as a food source, monitoring studies evaluating the MPs ingestion rates of common carp can give clues to the potential transfer of MPs to humans. Even though the danger of fish consumption due to MPs contamination is debatable, previous literature showed that necessary national and international measures need to be taken.

KAYNAKÇA

Atamanalp, M., Köktürk, M., Parlak, V., Ucar, A., Arslan, G., & Alak, G. (2022). A new record for the presence of microplastics in dominant fish species of the Karasu River Erzurum, Turkey. https://doi.org/10.1007/s11356-021-16243-w/Published

Chen, J., Rao, C., Yuan, R., Sun, D., Guo, S., Li, L., Yang, S., Qian, D., Lu, R., Cao, X. (2022) . Long-term exposure to polyethylene microplastics and glyphosate interferes with the behavior, intestinal microbial homeostasis, and metabolites of the common carp (Cyprinus carpio L.) Sci. Total Environ., 814,152681, https://doi.org/10.1016/J.SCITOTENV.2021.152681

FAO. (2022). Cyprinus carpio Linnaeus,1758. Fisheries and Aquaculture Division [online]. Rome. [Cited Monday, August 22nd2022] from: https://www.fao.org/fishery/en/aqspecies/2957/en

Fossi, M. C., Pedà, C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., & Baini, M. (2018). Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environmental Pollution, 237, 1023–1040. https://doi.org/10.1016/j.envpol.2017.11.019.

Jabeen, K., Su, L., Li, J., Yang, D., Tong, C., Mu, J., & Shi, H. (2017). Microplastics and mesoplastics in fish from coastal and fresh waters of China. Environmental Pollution, 221, 141–149. https://doi.org/10.1016/j.envpol.2016.11.055

Kılıç, E., Yücel, N., & Şahutoğlu, S. M. (2022). First record of microplastic occurence at the commercial fish from Orontes River. Environmental Pollution, 307, 119576.

Lee, B., Duong, C. N., Cho, J., Lee, J., Kim, K., Seo, Y., ... & Yoon, J. (2012). Toxicity of citrate-capped silver nanoparticles in common carp (Cyprinus carpio). Journal of Biomedicine and Biotechnology, 2012

M. Hamed, M., Monteiro, C.E., Sayed, A.E.D.H. (2022) Investigation of the impact caused by different sizes of polyethylene plastics (nano, micro, and macro) in common carp juveniles, Cyprinus carpio L., using multi-biomarkers Sci. Total Environ., 803,149921, https://doi.org/10.1016/J.SCITOTENV.2021.149921

Merga, L. B., Redondo-Hasselerharm, P. E., van den Brink, P. J., & Koelmans, A. A. (2020). Distribution of microplastic and small macroplastic particles across four fish species and sediment in an African lake. Science of the Total Environment, 741. https://doi.org/10.1016/j.scitotenv.2020.140527

Park, T. J., Lee, S. H., Lee, M. S., Lee, J. K., Lee, S. H., & Zoh, K. D. (2020). Occurrence of microplastics in the Han River and riverine fish in South Korea. Science of the Total Environment, 708. https://doi.org/10.1016/j.scitotenv.2019.134535

Rasta, M., Sattari, M., Taleshi, M. S., & Namin, J. I. (2021). Microplastics in different tissues of some commercially important fish species from Anzali Wetland in the Southwest Caspian Sea, Northern Iran.
Marine Pollution Bulletin, 169. https://doi.org/10.1016/j.marpolbul.2021.112479
Saad, D., Chauke, P., Cukrowska, E., Richards, H., Nikiema, J., Chimuka, L., & Tutu, H. (2022). First biomonitoring of microplastic pollution in the Vaal river using Carp fish (Cyprinus carpio)"as a bio-indicator". Science of The Total Environment, 836, 155623.
Scherer, C., Weber, A., Lambert, S., Wagner, M., 2018. Interactions of microplastics with FreshwaterBiotaEditors: Dami`a Barcel´o · Andrey G. Kostianoy. In: Freshwater Microplastics the Handbook of Environmental Chemistry, vol. 58, pp. 153–180. http://www.springer.com/series/698.
Turhan, D. Ö. (2022). Evaluation of microplastics in the surface water, sediment and fish of sürgü dam reservoir (Malatya) in turkey. Turkish Journal of Fisheries and Aquatic Sciences, 22(Special Issue). https://doi.org/10.4194/TRJFAS20157
Wardlaw, C. (2021). Microplastics in White Sucker (Catostomus commersonii) and Common Carp (Cyprinus carpio) from the Upper Thames River, Ontario. https://ir.lib.uwo.ca/etd
Zheng, K., Fan, Y., Zhu, Z., Chen, G., Tang, C., & Peng, X. (2019). Occurrence and Species-Specific Distribution of Plastic Debris in Wild Freshwater Fish from the Pearl River Catchment, China. Environmental Toxicology and Chemistry, 38(7), 1504–1513. https://doi.org/10.1002/etc.4437

INTRODUCTION AND PRODUCTIVITY OF *STYPHNOLOBİUM JAPONİCUM* (L.) SCHOTT STYPHNOLOBIUM JAPONICUM (L.) SCHOTT IN THE ABSHERON PENINSULA

S.B. Bagirova
M.Y. Hasanova
N.F. Shukurova
L.A. Atayeva
Institute of Dendrology, Azerbaijan, Mardakan settle., S.Yesenin str.89, Az1044

SUMMARY
The study will be conducted during 2020-2021 on the Absheron Peninsula, It was performed in the field of experience of the Institute of Dendrology of ANAS. *Fabales* or *Leguminosales* Bromhead as object of study. *Papilionaceae* or *Fabaceae* Lindl. one of the representatives included in the family *Styphnolobium* Scott. studies on the introduction of the *Styphnolobium japonicum* (L.) Schott species, included in the genus, were conducted.
From our studies, it has been found that most *Styphnolobium japonicum* seeds require similar conditions for germination. The percentage of germination of newly harvested seeds is 1.5-2.0 times higher than last year's seeds. Therefore, freshly collected *S. japonicum* seeds should be used during sowing.
From the morphological study of the sprouts, it was found that the life span of the cotyledons depends on the conditions under which the sprouts are cultivated and the biological characteristics of each species. Taking into account that the sown seeds give late or very weak (1-3%) sprouts, the seeds should be stratified at room temperature before sowing. In Absheron conditions, the best time to harvest fruits (seeds) of *S.japonicum* species is the end of November and December.
The results of the conducted researches showed that *S.japonicum* species introduced in Absheron's conditions have normal development stages and produce seeds with high germination capacity.
Keywords: *Styphnolobium japonicum, Fabaceae* Lindl., sprouts, introduction, productivity, germination percentage

LEUCAENA PULVERULENTA BENTH. TÜRÜNÜN TARIMDA EKONOMİK VERİMLİLİĞİ

ECONOMIC EFFICIENCY OF THE *LEUCAENA PULVERULENTA* BENTH. TYPE IN AGRICULTURE

Ph.D. Minare Hasanova
Orcid 0000-0001-5222-9366
Scientific secretary of the Institute of Dendrology of ANAS
Aynur Huseynova
Researcher of the Institute of Dendrology of ANAS
Leyla Atayeva
Orcid 0000-0002-1017-9468
Researcher of the Institute of Dendrology of ANAS
Saadat Aliyeva
Researcher of the Institute of Dendrology of ANAS
Nigar Badal-zade
Researcher of the Institute of Dendrology of ANAS

ÖZET

Tarım sektöründe yeni inovatif fikirlerin oluşması günümüzün güncel sorunları listesinde yer almaktadır. Tarımın geliştirilmesi ve ekolojik dengenin korunması doğrultusunda hem devlet politikasında hem de küresel ölçekte sürekli adımlar atılmaktadır. Bu gelişme, insan nüfusunun ihtiyaçlarını tam olarak karşılamalı ve aynı zamanda ekolojik dengenin korunmasında rol oynamalıdır. Talebin karşılanması, ekonomik açıdan günümüzün yoğun gelişimini ayaklaşmalı ve mali açıdan daha az maliyetli olmalıdır. *Leucaena* Benth cinsine ait *Leucaena pulverulenta* Benth. tarafımızdan araştırılmıştır. Çevresel değişikliklere hızlı adaptasyonu, kuraklığa ve hatta orman yangınlarına toleransı, Abşeron'un kuru-subtropikal ikliminde gelişme döngüsünü tam olarak tamamlayabilmesi, bu türün tarımda kullanılmasına, yeşilliklerin salınmasında ve toprak koruma örtülerinin kurulması yönelik çalışmalarda sürdürülübilir bir tür olarak ön plana çıkarmaktadır. İskender E.O.'nün "ileriye dönüklük ölçeği"nde 87 puan ile değerlendirilebilir. Yürütülen araştırmanın temel amacı *Leucaena pulverulenta* Benth'tir. tarımda ekonomik açıdan ucuz ve kaliteli, kullanımı kolay bir tür olarak yeşillendirmede yerel iklim koşullarına tam uyum sağlayan (beyaz başlı mimoza) türlerine öncelik verilmesidir. Bitki gen havuzunun artırılması ve doğal kaynakların verimli kullanılması yönünde türlerin tarımda kullanılması uygundur.

Anahtar sözcüklər: Tarım, bioekoloji özellikler, *Leucaena pilverulenta* Benth.

ABSTRACT

The formation of new innovative ideas in the agricultural sector is on the list of current problems of today. In the direction of the development of agriculture and maintenance of ecological balance, continuous steps are taken both in the state policy and on a global scale. This development should fully meet the needs of the human population and also play a role in maintaining the ecological balance. The satisfaction of the demand should surpass today's intensive development from the economic point of view, and it should be less costly from the financial point of view. *Leucaena* Benth, researched by us. belonging to the genus *Leucaena pulverulenta* Benth. the use of this species in agriculture, greening, and the establishment of soil protection covers brings it to the fore as a prospective species. Its quick adaptation to environmental changes, its tolerance to drought and even forest fires, its ability to fully complete its development cycle in the dry-subtropical climate of Absheron can be evaluated

with 87 points in E.O. Iskandar's "prospectivity scale". The main purpose of the conducted research *is Leucaena pulverulenta* Benth. in agriculture. (white-headed mimosa) species, which fully adapts to local climatic conditions in greening, as a cheap and high-quality, easy-to-use species from an economic point of view, was to be prioritized. It is appropriate to use the species in agriculture in the direction of increasing the plant gene pool and efficient use of natural resources.
Key words: agriculture, bioecological characteristics, *Leucaena pulverulenta* Benth.

INTRODUCTION

In modern times, the rapid development of agriculture has a great, destructive and irreversible effect on terrestrial natural ecosystems. The deepening of the degradation process in the ecosystem radically changes the forest landscape and leads to the simplification of nature. The creation of protected areas and the introduction of landscape protection species are considered an important resource in restoring the succession of natural groups. In addition to the natural plant gene pool, it is very important to introduce cultivated dendroflora and enrich it with new species resistant to soil and climate conditions. For the sustainability and longevity of green strips, it is advisable to use plants with more valuable decorative properties. Leucaena pulverulenta Benth. bioecological characteristics, generative reproduction, phenology, morphology, prospects of the species were investigated in Absheron conditions. It can be considered for use in decorative, fire and wind protection strips, medium-high protein value as fodder, in beekeeping as a fruit bearing species.

MORPHOLOGICAL AND PHYSIOLOGICAL ANALYSIS OF THE SPECIES (LEUCAENA PULVERULENTA BENTH.).

Leucaena pulverulenta Benth. is an evergreen shrub or tree with gray-brown bark up to 20 m tall [12]. The leaves are bipinnate, up to 30 cm long, consisting of 3-10 pairs of first-order leaves, and 10-20 pairs of second-order soft lanceolate leaves. The flowers are greenish-white with a diameter of about 2 cm. Fruits are flat-flat or slightly crescent-shaped pods up to 20 cm long and 1.5-2 cm wide, pointed at the end. The skin of ripe pods is thin, brown and brittle. Each pod contains 16-18 ovoid seeds, 6-10 mm in diameter, dark brown when ripe [2].

During stratification, a stress condition is created by soaking the hard shell of the seeds with very hot water (~80°C) and then placing them in cold water. It is kept in water for 12 hours and planted in wet soil under glass without drying.

Leucaena pulverulenta Benth. the seeds were sown outdoors and indoors, in the autumn season on 04.10.2020. During the study, the plant was sown at a depth of 1.5-2.0 cm both in ordinary soil and in a substrate prepared in the form of a mixture of soil, sand and peat (1:1:1) [3].

Figure 1. *Leucaena pulverulenta* Benth. in autumn. sprouts of the species in open and closed conditions.

The conclusion of the experiment showed that the development of plants is more intensive in the prepared soil mixture depending on the bioecological characteristics of the species. It is known that the organic and inorganic substances in the soil have a certain influence on its physical and chemical properties. During the research, it was concluded that the growth of plants is 1-3 times higher in the prepared soil mixture rich in organic matter.

When studying the morphological characteristics of the sprouts of the seeds sown in the fall in open and closed conditions, it was found that the sprouts of the Leucaena pulverulenta species are terrestrial and have high germination characteristics. Temperature has a two-way effect on plant seeds.

1. Low positive temperature awakens seeds from dormancy
2. Temperature ensures seed germination [14].

The first sprouts of the seeds sown in autumn (24.09.2020) were observed after 7-8 days, and mass sprouts after 10-13 days. 80-83% results were achieved (06.10.2020). When the seeds of the plant germinate, the upper part of the cotyledons remains inside the seed, which uses the seed as a nutrient to form the sprout that develops from the embryo. Petals are shiny, oval, obtuse, heart-shaped, light green in color, later darkening. Hypocotyl is white, 1.9-2.0 cm long, and epokotil is 1.5 cm. The development of the root system begins at the same time as the development of the above-ground part. The life of the leaves lasts 40 days. As true leaves develop, their margins become ciliate [4].

The seeds sown indoors grow well at 19-20°C. On 02.11.2020, the height of one-year Leucaena pulverulent species sown indoors was 19 cm, the length of the main root was 11 cm, the diameter of the root throat was 0.3 cm, and the length of the lateral roots was 6-8 cm [7]. The more intensive development of the root system of Leucaena pulverulenta is an adaptation for plants growing on dry stony, sunny slopes.

Figure 1. Study of the root system of the species

As the root system is formed, intensive growth of the above-ground part of the plant is observed, the root system is spindle-shaped. Such plant development is favorable for Absheron's dry climate. The root uses the underground water in the lower layers more efficiently by working deep. It is recommended to transfer the plant to the appropriate area no later than 2-3 years old. The dynamic development of the aerial parts is one of the main life indicators of the plant. The growth of Leucaena pulverulenta starts from the second decade of April and continues until the end of October. About 210+7 days of vegetation. The plant is warm and light-loving, its height is 2.5 m in 5-6 years, the diameter of its trunk is 14 cm, and the length of its side branches is 85-90 cm [6]. It bears fruit from 4-5 years of age.
In the conditions of Absheron, the beginning of the development phases of the annual *Leucaena pulverulenta* species and the duration of its completion were studied.

Table 1. *Leucaena pulverulenta* Benth. I annual development dynamics of the species (2020-2021)

Type	**Height (sm)**	**Annual height progress**		**Growing duration.**	**Annual height increase (sm)**
		begin.	**end.**		
L.pulverulenta Benth	23 ± 5	23.04.±1	29.10.±3	210±7	19 ± 5

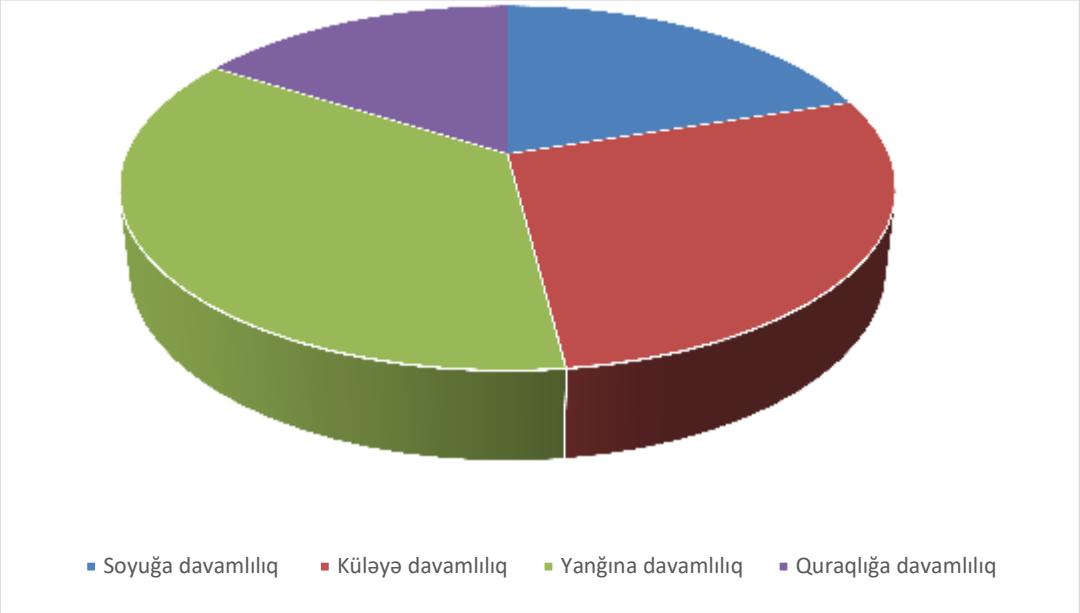
The requirements of *Leucaena pulverulenta* species for heat, light, moisture, cold air flow, and soil nutrition were studied in Absheron conditions. The results of the study are given in table 2.

Table 2. *Leucaena pulverulenta* Benth. the main environmental factors affecting the species

Type	**Environmental factors**				
	Heat	**Light**	**Humidity**	**Cold weather**	**soil**
Leucaena leucocephala	+	+	+	-	±

Note: (+) highly demanding, (-) low demanding, (±) relatively demanding. *Leucaena leucocephala* species is highly demanding towards ecological environmental factors during growth and flowering, fruit processing, and less demanding during rest period [5] .

Graph 1. *Leucaena pulverulenta* persistence indicators



The cold resistance of the plant depends on the external environmental conditions. When they are given potassium fertilizer, the cold resistance of their above-ground organs increases significantly as a result of the increase in the relative humidity of the air.

EVALUATION OF THE PROSPECTIVITY OF THE SPECIES (LEUCAENA PULVERULENTA BENTH.).

The efficiency of the use of nature is based on economic, cultural and healthy effects. In order to protect the environment and improve ecological activities, the state implements a system of various measures. In order to ensure environmental safety, their economic activity is the main influencing factor in people's health [13].

The problem of desertification in modern times is a global problem. Desertification is caused by disturbance and destruction of vegetation, excessive livestock grazing, non-observance of agrotechnical rules. According to the judgments issued based on the results of the conference "Combating desertification and drought in the 21st century" held in Rio de Janeiro, according to the clause "the use of drought-resistant fast-growing native and other introducers", the mentioned species fully meets all the requirements [10].

The species is resistant to frost and fire. If the above-ground part is destroyed by frost, a new plant grows from its root in the second year. In tropical humid or semi-arid climates, with an average annual rainfall of 650 to 3000 mm, it tolerates drought of 6-7 degrees. Development at temperatures between 35 and 38 °C stops growing in cold periods. Leucaena pulverulenta Benth. it is not completely destroyed during forest fires, the surviving roots regenerate after burning. After cutting branches or leaves, leaves regenerate very quickly [13]. Hard seeds do not lose their quality for up to 20 years. Under ideal conditions, with sufficient heat and water, it blooms all year round and fruits ripen 10-15 weeks after flowering, the plant can bear fruit in the first year after planting. This species has a symbiotic relationship with soil bacteria of the genus Rhizobium, which live in root nodules and fix atmospheric nitrogen, part of which is used by the plant, and the rest enriches the soil. Deep rooting helps break up compacted subsoil, thus improving water penetration and reducing surface runoff. Saline prevents groundwater from coming to the surface. Requires a full sun site with calcareous sandy soil up to pH 8. Leucaena pulverulenta, which grows in contour strips, helps to control erosion on steep slopes. It prevents wind damage, and its leaves are used as green manure [8], [9].

Table 3. Evaluation of the prospects according to the indicators of vitality

№	Type	Age of plant	form	Vital indicators								Genereal evaluation	
				Woodening of sprout	Drought resistance	Cold resistance	Maintenance of habitus	Arousal formation	Growth	Generative reproduction	Reproduction in culture	The sum of vital signs	Prospective group
1.	*Leucaena pulverulenta* Benth.	10	ağac	10	20	5	5	5	10	25	7	87	II

When assessing the prospectiveness of the introduced species, we used E.O. Iskandar's "Prospectiveness assessment" scale [11]. The viability of the species was calculated taking into account the opposite effects of the plant in Absheron stress. So, it is clear from the division that the highest generative reproduction is given. Because the main form of reproduction of Leucaena pulverulenta is by seed. Calculations taking into account the age of the plant is an important factor. The age of old and young individuals can cause the plant to change its perspective value. We considered groups IV:

- High vitality indicator of the type we included in group I (91-100 points);
- Average prospective for group II (71-90 points);
- Group III weak perspective (51-70);
- IV group without prepective

Leucaena pulverulenta included in group II was evaluated between 81-90 points according to vitality indicators.

THE IMPORTANCE OF THE SPECIES (LEUCAENA PULVERULENTA BENTH.) IN FOOD AND AGRICULTURE

Young shoots, young leaves and seeds can be used as vegetables in human nutrition. In the Philippine Islands, the young pods are cooked as a vegetable and the seeds are used as a coffee substitute. Ripe seeds are sometimes eaten dried like popcorn. Leucaena has a high honey yield, which allows it to be used in beekeeping. It is valued as an excellent source of protein for cattle feed, whether mature or immature, consumed green or dry. Nutritive value equals or exceeds alfalfa. The wood is hard and heavy (sp. gr. 0.7), used for fuel or charcoal. It is used in making small furniture and paper pulp. It is used in various jewelry for tourists in Puerto Rico and the Virgin Islands. The seeds and young leaves contain 4% mimosine, which causes shedding in ruminants, especially ungulates. It has high antioxidant properties. The extract of the seeds has anthelmintic properties.

THE RESULT

The plant is warm and light-loving, its height is 2.5 m in 5-6 years, the diameter of its trunk is 11 cm, and the length of its side branches is 85-90 cm. It bears fruit from 4-5 years of age. From the results of the research, it was found that although the seeds sown in both conditions (open and closed) give good results, it is more appropriate to sow the seeds in closed conditions in autumn. Because cold weather is unfavorable for the development of seeds sown

in open conditions. After three years, the plant can be transplanted to permanent selected outdoor conditions.

Withstands short-term temperature drops up to -6 C. Leucaena pulverulenta Benth. growth of the species starts from the second decade of April and continues until the end of October. About 210+7 days of vegetation.

Tolerant to the change of rainy and dry seasons, thermophilic, does not like soils with high acidity and aluminum content.

Leucaena pulverulenta Benth. both economic and environmental protection are fully responsive to the measures taken in the direction. Leucaena pulverulenta Benth, which we studied under Absheron conditions. during the vegetation period, all the development phases of the species pass normally, and it can complete its cycle. The ability to self-regenerate and produce quickly allows this species to be easily propagated and used in living hedges.

The nutritional importance of its tissues, its use in dry and wet mass for cattle feed, and the ability to obtain high yields make the species promising.

LITERATURE

1. Agamirov U.M., Kurbanov M.R. (1985) History of the introduction of ornamental tree plants to Apsherone // Труды Бот. Sada Inst. Botany AN. Azerb. SSR "Introduction and acclimatization of plants". Baku, Elm, pp. 18-21.
2. Sokolova I. B.(2014) // Papaya / П — Perturbation function. — M. : Big Russian encyclopedia, C. 248-249.
3. Firsova M.K. Methods of research and quality evaluation of seeds / M.K. Firsova. - Moscow: Selkhozgiz, -1955. -376 p.
4. Serebryakov I.G. Morphology of vegetative organs of higher plants / I.G. Serebryakov - Moscow: Sovetskaya nauka, -1952, -p.140-143.
5. Iskenderov E.O. (1989) Study of the bioecological features of some rare and disappearing woody plants of the Caucasus on Apsherone. Dis...kand.biol.nauk. Baku, 248 p.
6. Molchanov A.A. Method of studying the growth of tree plants / A.A. Molchanov, V.V. Smirnov - Moscow: Nauka, -1967. -100 c.
7. Kolesnikov V.A. Methods of studying the root system of tree plants / V.A. Kolesnikov - Moscow: Lesn. industry, -1972. -152 c.
8. On the conditionality of specifying the class of dicotyledons as a higher taxon for the group of plants described in this article, see section «APG systems» articles «Double-sided».
9. Species of Leucaena submitted to The Plant List (eng.) (lat.) (Data update: May 25, 2013)
10. MammadovGarib, Khalilov Mahmud; "Ecology, environment and man", Baku-Science-2006; page 182
11. Gurbanov.M.R., Iskander E.O.; "Bioecology, propagation and protection of rare woody plants of Azerbaijan", Baku-2015; page 169
12. Mammadov T.S. "Dendroflora of Azerbaijan" Volume III, Baku-Sada-2015, page 65
13. Mammadov T.S. "Ecology", Baku-Elm-2003, page 200
14. Mammadov T.S., Asadov H.H. "Plant Ecology" Baku-Elm-2014, page 29

SOĞAN VE SARIMSAKTAKİ LEZZET BİLEŞENLERİ
FLAVOR COMPOUNDS IN ONION AND GARLIC

Dr. Emine NAKİLCİOĞLU
Ege University, Engineering Faculty, Food Engineering Department

ÖZET
Gıdaların tüketiciler tarafından tercih edilmelerini etkileyen en önemli faktörlerden biri, lezzetleridir. Lezzet, genellikle tat ve kokudan oluşmasının yanı sıra acı ve sıcaklık uyarılarını da içeren karmaşık bir olgudur. Gıdaların duyusal bir karakteristiğidir ve ağızda oluşan duyuların toplamıdır. Lezzet bileşenleri, ürünün kalitesini ve tercihini yakından etkilemektedir ve gıda işleme yöntemlerinden etkilenebilmektedir. Soğan ve sarımsak, Dünya mutfağının vazgeçilmez gıdalarıdır. Her ikisi de Alliaceae familyasının Allium cinsine aittir ve hem besin maddesi hem de ilaç hammaddesi olarak kullanılmaktadır. Yapılarındaki antioksidan özellik gösteren fitokimyasallar sebebiyle kanser, kardiyovasküler hastalıklar, diyabet, obezite, hipertansiyon ve katarakt gibi hastalıkların önlenmesinde ve tedavisinde öne çıkmaktadırlar. Bu fitokimyasallar, uçucu bileşenler olarak sülfürlü bileşikler ve uçucu olmayan bileşenler olarak da fenolik bileşikler şeklinde sınıflandırılabilir. Bunların arasında sülfürlü bileşikler, soğan ve sarımsağın lezzetine doğrudan etki eden bileşikler olarak bilinmektedir. Çiğ olarak tüketilen soğan ve sarımsak acımsı bir lezzete sahiptir. Bu acımsı lezzetten, sülfürlü bileşikler sorumludur. Şaşırtıcı bir şekilde soğanı kavurduğumuzda bu acı lezzetin yerini tatlı bir lezzetin aldığı görülmektedir. Burada sülfürlü bileşiklerin uzaklaşmasıyla birlikte yapıdaki şeker bileşenlerinde karamelizasyon meydana gelmesi etkilidir. Bu çalışmada çiğ soğan ve sarımsağın sahip olduğu acımsı lezzetten ve soğanın pişirildiğinde tatlı lezzete sahip olmasından sorumlu olan bileşenler ve pişirme işleminde meydana gelen, lezzeti yakından etkileyen mekanizmalar hakkında bilgi verilmesi amaçlanmıştır.
Anahtar Kelimeler: Karamelizasyon, sarımsak, soğan, sülfürlü bileşenler.

ABSTRACT
One of the most important factors affecting food preference by consumers is the taste of foods. The flavor is a complex phenomenon, often consisting of taste and odor, as well as stimuli of bitterness and temperature. It is a sensory characteristic of foods and is the sum of the sensations that occur in the mouth. Flavor components closely affect the quality and preference of the product and can be affected by food processing methods. Onion and garlic are indispensable foods of world cuisine. Both of them belong to the Allium genus of the Alliaceae family and are used as both food and pharmaceutical raw materials. They come to the forefront in the prevention and treatment of diseases such as cancer, cardiovascular diseases, diabetes, obesity, hypertension and cataracts due to their antioxidant properties. These phytochemicals can be classified into sulfurous compounds as volatile compounds and phenolic compounds as non-volatile compounds. Among them, sulfur compounds are known as compounds that directly affect the flavor of onions and garlic. Onions and garlic consumed raw have a bitter taste. Sulfur compounds are responsible for this bitter taste. Surprisingly, when we roast the onion, it is seen that this bitter flavor is replaced by a sweet flavor. Here, the caramelization of the sugar components in the structure and the removal of the sulfur compounds are effective. In this study, it is aimed to give information about the components responsible for the bitter taste of raw onions and garlic and the sweet taste of onions when cooked, and the mechanisms that occur in the cooking process and closely affect the flavor.
Keywords: Caramelization, garlic, onion, sulfur compounds.

1. GİRİŞ

Soğan (*Allium cepa* L.), tipik keskin aroması, güçlü tadı ve göz yaşartıcı etkileri nedeniyle dünya çapında en popüler sebzelerden biridir. Bu duyusal özellikleri sayesinde soğan, neredeyse tüm ulusal mutfakların birçok yemeğinin önemli bir bileşenidir. Gıda endüstrisinde baharat, çorba, konserve ve turşu, sosis ve diğer et ürünleri, salata, ketçap ve sos gibi çok sayıda ürünün üretiminde önemli bir rol oynamaktadır (Kubec ve ark., 2018).

Sarımsak (*Allium sativum* L.), Alliaceae familyasına ait ve geleneksel yemeklerde yaygın olarak kullanılan bir ingredienttir. Ayrıca sarımsak, güçlü tıbbi özelliklere sahiptir. İnsan sağlığı üzerine olumlu etkileri vardır. Metabolik ve kardiyovasküler rahatsızlıklar gibi farklı hastalıkları önlemek için kullanılır. Yemeklerde ve tıpta kullanımı, organoleptik özelliklerine bağlıdır (Martínez-Casas ve ark., 2017).

2. SOĞAN VE SARIMSAĞIN YAPISINDAKİ SÜLFÜRLÜ BİLEŞİKLER

2.1 ÇİĞ SOĞAN VE SARIMSAK'A ACILIK VEREN LEZZET BİLEŞENLERİ

Çiğ soğan ve sarımsağın acı bir lezzete sahip olmasının sebebi, yapılarındaki sülfürlü (kükürtlü) bileşiklerdir.

Soğandaki ısı ve acı hissinden sorumlu olan bileşik, propanetial-S-Oksit (PSO)'tir. Propanetial-S-oksit (C_3H_6OS), tiyokarbonil-S-oksit (sülfinler) olarak bilinen organosülfür bileşikler sınıfının bir üyesidir ve gözle temas ettiğinde göz yaşı oluşturucu ajan olarak görev yapar. Soğan dilimlendiği zaman, hücrelerden alliinazlar adı verilen enzim salınır ve aminoasit sülfoksitleri parçalayarak sülfonik asitleri oluşturur. Soğanlar kesildiğinde oluşan spesifik bir sülfenik asit olan 1-propensülfenik asit, gözyaşı faktörü sentezleyicisi (LFS) adı verilen ikinci bir enzim tarafından hızlıca yeniden düzenlenir ve sin-propanetial S-oksit meydana gelir. Bu uçucu bileşik havada yayılır ve gözle temas ettiğinde, acı bir his yaratan duyusal nöronları uyarır. Tahriş edici maddeyi seyreltmek ve dışarı atmak için gözyaşı bezlerinden gözyaşı salınır (Anonim, 2021a).

Sarımsakta bulunan ve acı algısını harekete geçiren bileşik ise Allisin'dir. Alliin ((+)-S-allil-L-sistein sülfoksit) ve allisin (S-(+)-2-propenil-L-sistein sülfoksit), sarımsakta bulunan iki önemli kükürt içeren bileşiktir. Hem alliin hem de allisin, organosülfür bileşikleri olarak bilinir. Sarımsak kesildiğinde veya ezildiğinde, sarımsak zarı bozulur ve alliin, allinaz tarafından enzimatik olarak allisine dönüştürülür. Bir diş sarımsağın ezilmesiyle allinin biyolojik olarak aktif allisin molekülüne dönüşümü çok hızlıdır ve saniyeler içinde tamamlanır. Sarımsak ezilene veya işlenene kadar kokusuzdur. Ezilme ile ortama salınan allisin uçucu bir bileşiktir. Sarımsağın kendine özgü acımsı tadından ve kokusundan sorumludur (Touloupakis & Ghanotakis, 2010).

2.2 PİŞİRİLMİŞ SOĞAN'IN TATLI LEZZETE SAHİP OLMASININ NEDENLERİ

Soğan, pişirme işlemine tabi tutulduğunda tatlı bir lezzete sahip olmaktadır. Bunun ilk sebebi, sülfürlü bileşiklerin ısıyla buharlaşmasıdır. Tatlılık, soğan lezzetinde önemli faktörlerden biridir. Şekerler, soğanın çözünür katılarının büyük bir bölümünü oluşturur, ancak aromada sülfürlü uçucu bileşiklerinin aşırı baskın olması nedeniyle, bunların varlığı, çiğ soğanda tespit edilemez. Üç ana şeker bileşeni olan fruktoz, glikoz ve sakkarozun yanında çözünebilir kompleks karbonhidratlardan fruktanın miktarı, soğanın kuru ağırlığının %65 veya daha fazlasından sorumludur. Soğanın tatlı tadını algılayabilmek için pişirme işlemi uygulanır. Pişirme işlemi ile keskin tada sahip uçucu sülfürlü bileşikler buharlaştırılmakta ve ısıl işlemle herhangi başka bir enzim etkisi engellenmektedir. Böylece pişirilmiş soğanın tadı, tatlı olarak algılanmaktadır (Crowther ve ark., 2005).

Diğer sebebi ise şekerlerin karamelizasyonudur. Karamelizasyon, gıdalarda esmerleşme işleminin en önemli tiplerinden biridir. Gıdalarda sadece renkte değil aynı zamanda lezzette de önemli değişiklere neden olur. Karamelizasyon işlemi, enzim içermediği için enzimatik olmayan esmerleşme reaksiyonudur. Karamelizasyon, yüksek konsantrasyonlarda

karbonhidrat (şeker) içeren gıdalara ısıl işlem uygulanması süresince meydana gelir. Çiğ soğanın tadı, muhtemelen keskinlik algısı nedeniyle tatlı bir tada sahip değildir. İngiltere'de yemeklik soğan olarak satılan Rijnsburger tipi soğanlarda ağırlıkça % 8-10 oranında şeker bulunur, ancak tatlı olarak algılanmazlar. Karamelizasyon işlemi şekerin yüksek sıcaklıklarda erimesi ile başlar, bunu köpürme (kaynama) takip eder. Bu aşamada sakkaroz, glukoz ve fruktoza parçalanır. Bunu, şekerlerin su kaybettiği ve diğerleri ile tepkimeye girdiği kondensasyon basamağı izler, örneğin difruktoz-anhidrin oluşur. Bir sonraki basamak, aldozların ketozlara izomerizasyonu ve daha ileri dehidrasyon reaksiyonlarıdır. Son reaksiyon dizisi, parçalanma reaksiyonlarını (lezzetin üretimi) ve polimerizasyon reaksiyonlarını (renk üretimi) içerir. Karamelizasyon reaksiyonları sonucunda lezzet bileşenleri de oluşur. Diasetil önemli bir lezzet bileşiğidir; karamelizasyonun birinci basamağında üretilir. Diasetil, çoğunlukla tereyağı lezzetinin oluşumundan sorumludur. Bundan başka; furanlara örnek olarak hidroksimetilfurfural (HMF) ve hidroksiasetilfuran (HAF); fronanlara, hydroksidimethilfuranone (HDF), dihidrosidimetilfuranon (DDF) ve disakkaritlerden maltol ve monosakkaritlerden hydroksimaltol oluşabilir. Hidroksimetilfurfural (HMF) balda, meyve sularında, sütte hatta sigarada bile bulunur. Hidroksiasetilfuran (HAF), tatlı bir aromaya sahiptir ve düşük koku eşiğindedir. Maltol, taze fırın ekmeğini hatırlatan tada sahiptir, ekmeklerde ve keklerde lezzet attırıcı (E636) olarak kullanılır (Anonim, 2017). Soğan gibi doğal şekerler açısından zengin sebzeler pişirilirken, yapılarındaki poli- ve disakkaritler daha küçük şeker birimlerine parçalanır, çünkü birimler arasındaki bağlar ısı ile bozulur. Isıtma devam ederken, bu şeker bileşikleri karamelizasyona uğrar. Kuru ısıtmada soğanların kahverengiye dönüşmesinin ve daha tatlı bir lezzet geliştirmesinin arkasındaki nedenlerden bir diğeri de budur (Özilgen, 2019).

SONUÇ

Soğan ve sarımsağın, insan beslenmesindeki önemi ve sağlığa faydalı özelliklerinin yanı sıra eşsiz tat ve aromaya sahip olmaları, dünyadaki tüketimlerini giderek arttırmaktadır. Soğanın kendine özgü tat ve aroması, uçucu olan ve uçucu olmayan bileşenlerden kaynaklanmaktadır. Bunlar arasında uçucu bileşenlerden olan sülfürlü bileşikler öne çıkmaktadır. Çiğ halde tüketilen soğan ve sarımsağın acı lezzetinden sülfürlü bileşikler sorumludur. Soğanın pişirilmesiyle bu acı lezzetin ortadan kalktığı, hatta tatlı bir lezzetin oluştuğu dikkatleri çekmektedir. Bunun sebebi ise sülfürlü bileşiklerin buharlaşması ve şekerlerin karamelizasyonudur. Gıdaların lezzet bileşenlerinin belirlenmesi ve mekanizmalarının aydınlatılması, görüldüğü gibi, oldukça önemlidir. Gıda işleme sırasında istenilen kalitede ve istenilen tat ve aromada ürünleri elde edebilmek için lezzet bileşenleri ve gıda işleme ile meydana gelen değişim mekanizmaları hakkında daha ayrıntılı çalışmalara gereksinim duyulmaktadır.

KAYNAKÇA

Anonim. (2017). Karamelizayon. http://www.food-info.net/tr/colour/caramel.htm (Erişim tarihi: 24.08.2021).

Anonim. (2021a). Syn-Propanethial-S-oxide. https://en.wikipedia.org/wiki/Syn-Propanethia l-S oxide#:~:text=syn%2DPropanethial%20S%2Doxide%20(,cepa%2C%20as%20they%20are%20sliced (Erişim tarihi: 24.08.2021).

Crowther, T., Collin, H. A., Smith, B., Tomsett, A. B., O'Connor, D., & Jones, M. G. (2005). Assessment of the flavour of fresh uncooked onions by taste-panels and analysis of flavour precursors, pyruvate and sugars. Journal of the Science of Food and Agriculture, 85(1), 112-120.

Kubec, R., Štefanová, I., Moos, M., Urajová, P., Kuzma, M., & Zápal, J. (2018). *Allithiolanes*: nine groups of a newly discovered family of sulfur compounds responsible for the bitter off-taste of processed onion. Journal of Agricultural and Food Chemistry, 66(33), 8783-8794.

Martínez-Casas, L., Lage-Yusty, M., & López-Hernández, J. (2017). Changes in the aromatic profile, sugars, and bioactive compounds when purple garlic is transformed into black garlic. Journal of Agricultural and Food Chemistry, 65(49), 10804-10811.
Özilgen, S. (2019). Cooking as a chemical reaction: culinary science with experiments. CRC Press.
Touloupakis, E., & Ghanotakis, D. F. (2010). Nutraceutical use of garlic sulfur-containing compounds. In Bio-Farms for Nutraceuticals (pp. 110-121). Springer, Boston, MA.

GIDALARDA POLİFENOL OKSİDAZ (PPO) ENZİMİ VE İNHİBİSYON YÖNTEMLERİ

POLYPHENOL OXIDASE (PPO) ENZYME AND INHIBITION METHODS IN FOODS

Dr. Emine NAKİLCİOĞLU
Ege University, Engineering Faculty, Food Engineering Department

ÖZET

Bakır içeren bir metalloprotein olan polifenol oksidaz, meyve ve sebzelerde bulunan fenolik bileşiklerin, ilk aşamada kinonlara oksidasyonunu katalize etmektedir. Bu reaksiyonun gerçekleşmesi için ürünün kesme, dilimleme ve kabuk soyma gibi bir mekanik işleme tabi tutulması ya da meyve/sebzenin olgunlaşması sonucu hücre yapısının değişmesi gerekmektedir. Böylece serbest hale geçen polifenol oksidaz enzimi ile fenolik bileşikler reaksiyona girmekte ve önce kinonlara daha sonra ise kinonlar polimerleşerek melanin adı verilen kahverengi, kırmızı veya siyah pigmentlere dönüşmektedir. Bu enzimatik mekanizma, hasat sonrası kayıplara neden olmaktadır. Ürünlerin renkleri başta olmak üzere, lezzetlerinde ve kalitelerinde düşüşler meydana gelmektedir. Kalite kayıplarını önlemek adına, gıdaların yapısında bulunan polifenol oksidaz enziminin inhibe edilmesi gerekmektedir. Bu amaçla gıda sanayinde geleneksel suda haşlama, buharlı haşlama, ohmik ısıtma, yüksek nemli sıcak hava haşlaması, mikrodalga ısıtma, gamma ışınlaması, yüksek basınç uygulaması, süperkritik karbondioksit uygulaması, vurgulu elektrik alan uygulaması, ultrason uygulaması ve enzimatik esmerleşme karşıtı kimyasal ajan kullanımından faydalanılmaktadır. Bu çalışmada polifenol oksidaz enziminin bazı özellikleri üzerinde durulmuş, enzimatik esmerleşme reaksiyonuna katkısı ve reaksiyonun oluşum mekanizmaları incelenmiş ve bu enzimi inhibe etmek için kullanılan geleneksel ve alternatif yöntemler hakkında bilgiler sunulmuştur. Polifenol oksidazın enzimatik esmerleşme mekanizmasındaki etkisinin anlaşılmasının, meyve ve sebzelerin esmerleşmesini kontrol etmek için daha etkili yöntemlerin geliştirilmesine veya seçilmesine yardım edeceği düşünülmektedir.

Anahtar Kelimeler: Fenolik bileşikler, inhibisyon, ohmik ısıtma, polifenol oksidaz, sülfitler.

ABSTRACT

Polyphenol oxidase, a copper-containing metalloprotein, catalyzes the oxidation of phenolic compounds in fruits and vegetables to quinones in the first step. For this reaction to occur, the product must be subjected to a mechanical process such as cutting, slicing, and peeling, or the cell structure must change due to the fruit/vegetable ripening. Thus, the free polyphenol oxidase enzyme reacts with phenolic compounds and first quinones are formed, and then the quinones polymerize and turn into brown, red or black pigments called melanin. This enzymatic mechanism causes post-harvest losses. There is a decrease in the taste and quality of the products, especially the color. In order to prevent quality losses, the polyphenol oxidase enzyme in the structure of foods should be inhibited. For this purpose, traditional boiling in water, boiling in steam, ohmic heating, High-humidity hot air impingement blanching, microwave heating, gamma irradiation, high-pressure processing, supercritical carbon dioxide, pulsed electric field, ultrasound application, and the use of enzymatic anti-browning chemical agents are utilized in the food industry for this purpose. In this study, some properties of the polyphenol oxidase enzyme are emphasized, its contribution to the enzymatic browning reaction and the formation mechanisms of the reaction are examined, and information about the traditional and alternative methods used to inhibit this enzyme is

presented. Understanding the effect of polyphenol oxidase on the enzymatic browning mechanism will help develop or select more effective methods to control the browning of fruits and vegetables.
Keywords: Phenolic compounds, inhibition, ohmic heating, polyphenol oxidase, sulfites.

1. GİRİŞ

Taze sebze ve meyvelerin sağlık yararları, yapılarında yüksek miktarlarda bulunan vitamin, lif ve antioksidanlar gibi biyoaktif bileşenlerden kaynaklanmaktadır. Antioksidan özelliğe sahip bileşikler hasat, depolama ve işleme aşamalarında değişikliğe uğrarlar (Yılmaz ve Elmacı, 2018). Öte yandan antioksidanlar, gıdalarda oksidatif bozulmayı önleyen ya da geciktiren bileşiklerdir. Oksidasyona bağlı olarak gelişen reaksiyonların sonucunda meydana gelen kötü koku ve lezzeti engelleyebilmektedirler. Bazı gıdaların yapısında doğal olarak antioksidan maddeler bulunurken, bazılarına da ekleme yapılabilmektedir. Gıdalara antioksidan madde ilave etmenin temelde iki sebebi bulunmaktadır. Bunlardan ilki, lipid oksidasyonunu önlemek ve uzun depolama ya da ısıtma koşullarında gıdalarda serbest radikallerin oluşumunun önüne geçmektir. İkincisi ise, gıdaların tüketiminden sonra oluşabilecek serbest radikallerin konsantrasyonlarını düşürmektir.

Gıda sanayinde ticari olarak en sık kullanılan sentetik antioksidanlar bütillenmiş hidroksi anizol (BHA), bütillenmiş hidroksi toluen (BHT), tersiyer bütil hidrokinon (TBHQ) ve propil galat (PG)'tır. Fakat tüketicilerin sentetik katkı maddeleri hakkındaki olumsuz yargıları ile minimum işlem görmüş, güvenli ve uzun raf ömrüne sahip gıdalara olan artan talep sonucunda, gıda muhafaza yöntemlerinde doğal antioksidanların kullanımına yönelinmektedir (Nakilcioğlu-Taş, 2016).

Sebze ve meyvelerin yapısında bulunan fenolik bileşikler, doğal antioksidan madde özelliğine de sahiptirler. Fenolik bileşikler sebze ve meyvelerde lezzetin oluşumunun yada sarı, sarı-esmer, kırmızı-mavi tonlardaki renklerin meyda gelmesinde de görev almaktadırlar. Sebze ve meyvelerin işlenmesinde ise enzimatik esmerleşme gibi istenmeyen renk değişimlerine neden olmaktadırlar. Gıda sanayinde depolama süresince meyve ve sebze kayıplarının en önemli nedenlerinden biri, enzimatik esmerleşmedir. Bu tip esmerleşmeye neden olan enzimler ise polifenol oksidazlar (PPO) olarak bilinmektedir (Yılmaz ve Elmacı, 2018). Bu çalışmada PPO enzimi, enzimatik esmerleşme mekanizması ve gıdalarda polifenol oksidazların inhibe edilerek enzimatik esmerleşmenin önlenmesi hakkında bilgi sunumu yapılmıştır.

2. POLİFENOL OKSİDAZLAR

Polifenol oksidazlar (E.C.1.10.3.1.) meyve ve sebzelerde enzimatik esmerleşmeden sorumlu olan metaloenzimlerdir (Gülsoy ve ark., 2019). Bitkinin hastalıklara karşı direncinin artmasını sağlamaktadırlar (Dedeoğlu, 2009). Aktif merkezlerinde bakır bulundurmakta ve oksidoredüktaz sınıfında yer alan enzimlerdendir (Gülsoy ve ark., 2019). Ayrıca bu enzimler tirozinazlar, krezolazlar, katekolazlar ve fenolazlar olarak da isimlendirilmektedirler (Cerrahoğlu ve Arabacı, 2016). PPO enzimi meyve ve sebzelerin yanı sıra kabuklu deniz ürünlerinde ve bazı hayvansal organlarda da bulunmaktadır. PPO enzim içeriği, bitkinin türüne ve yetiştirilme koşullarına bağlı olarak değişiklik gösterebilmektedir. Hatta meyve ve sebzelerin olgunluk seviyelerine göre enzimin bitki hücrelerindeki yerleşiminde farklılıklar gözlenmektedir. Meyvelerde, sebzelerde ve kabuklu deniz ürünlerinde, mekanik işlemlerden sonra esmerleşme adı verilen renk değişimleri görülmektedir (Ergün ve Çiçek, 2018). Enzimatik esmerleşme reaksiyonları, dört temel aşamadan oluşmaktadır. İlk önce özellikle kayısı, elma, armut, patates gibi açık renkli meyve ve sebzelerin dokularındaki fenolik maddeler (mono ve o-difenoller vb.), PPO enzimi katalizörlüğünde o-kinonlara dönüşmektedir. Oluşan o-kinon veya o-difenol, hidrosilizasyona uğrayarak trihidroksi benzen oluşmaktadır. Daha sonra o-kinon ve trihidroksi benzen tepkimeye girerek hidrokinonlar meydana gelmektedir. Son aşamada hidrokinonlar polimerize olmakta ve kırmızı-kahve, koyu

kahve hatta siyah renkli melanin adı verilen bileşikler oluşmaktadır. Böylece baştaki açık renkli gıdalarda esmerleşme gözlenmektedir. Enzimatik esmerleşme, fenolik bileşiklerin PPO enzimleriyle oksitlenmesi şeklinde özetlenebilir (Numanoğlu ve Çelik, 2018). Enzimatik esmerleşme reaksiyonlarında meydana gelen kinonlar, hücreler için toksik özelliğe sahiptir ve reaktif oksijen türlerinin oluşumuna sebep olmaktadırlar. Özellikle hasat sonrası taze meyvelerin depolanması sırasında, PPO enzimi kaynaklı esmerleşme reaksiyonları gözlemlenmektedir. Bunun sonucunda gıdanın görünüş, renk ve lezzet gibi organoleptik özellikleri ile besin değeri değişmektedir. Dolayısıyla ürünün kalitesinde ve ekonomik değerinde düşüş meydana gelmektedir. Aslında PPO enzimi ile fenolik bileşikler bir arada bulunmamaktadır. PPO, hücre içerisinde kloroplastlarda tilakoid mebrana bağlı durumda ya da stoplazmada bulmaktadır. Substratı olan fenolik bileşikler ise kofullarda yer almaktadır (Gülsoy ve ark., 2019). Meyvelerde, sebzelerde ve kabuklu deniz ürünlerinde enzimatik esmerleşmenin meydana gelmesi için kesme, dilimleme ve kabuk soyma gibi mekanik işlemlere ya da meyve ve sebzenin olgunlaşması sonucu hücre yapısının değişmesine bağlı olarak enzimin hücre dışına çıkmasına ihtiyaç vardır (Cerrahoğlu ve Arabacı, 2016; Gülsoy ve ark., 2019). Esmerleşme, fenolik bileşiklerle PPO enzimnin, oksijen, pH, sıcaklık ve su aktivitesi açısından uygun koşullar varlığında tepkimeye girmesi sonucunda oluşmaktadır (Cerrahoğlu ve Arabacı, 2019). Enzimatik esmerleşme ilerledikçe, belirli bir noktadan sonra durdurulamaz hale gelebilmektedir. Gıdanın rengiyle birlikte tadını ve kalitesini de bozmaktadır. İstenmeyen türden olan bu reaksiyonların önüne geçebilmek için PPO enziminin inhibe edilmesi, aktivitesinin sınırlandırılması veya tamamen durdurulması gerekmektedir (Ergün ve Çiçek, 2018).

3. GIDALARDA POLİFENOL OKSİDAZLAR'I İNHİBE EDEN YÖNTEMLER

PPO enziminin inhibe edilmesi üzerine çeşitli uygulamalar bulunmaktadır. Esmerleşmeyi engelleyen ajanların kullanılması, oksijenin uzaklaştırılması ve kimyasalların eklenmesi kullanılan en etkin metodlardandır. Gıda sanayinde PPO enziminin termal yolla inhibisyonu sayesinde enzimatik esmerleşmenin engellenmesi mümkündür (Cerrahoğlu ve Arabacı, 2016). Ama geleneksel ısı uygulamaları (suda haşlama, buharlı haşlama, yüksek nemli sıcak hava haşlaması, ohmik ısıtma) tat ve renk bozulmaları, vitamin kayıpları, besin değeri azalmaları gibi yan etkilere sebep olabilmektedir (Cerrahoğlu ve Arabacı, 2016; Yılmaz ve Elmacı, 2018). Enzimatik esmerleşme reaksiyonunu katalizleyen oksijen, meyve ve sebzelerin şurup, su veya tuzlu suya daldırılması ya da vakumlanması sonucunda ortamdan uzaklaştırılabilmektedir. Fakat bu tür uygulamaların hiçbiri kesin sonuç değildir. Örneğin, vakumlanan paketlerin açılmasıyla oksijen tekrar içeri girmekte ve esmerleşme reaksiyonu yeniden gerçekleşmektedir. Gıda sanayinde esmerleşme karşıtı ajanların kullanımına ürünün renk, tat, lezzet, doku ve maliyet üzerine etkileri ve toksisite yaratabileceği ihtimalinden dolayı tedirginlikle yaklaşılmaktadır. Bu bağlamda bisülfitler, askorbik asit ve analogları gibi enzimatik esmerleşmeyi engellemek amacıyla kullanılabilmektedir. Esmerleşmenin kontrol edilmesinde kullanılan en etkin yöntem, süfitlerin (kükürtdioksit, sodyum/potasyum metabisülfit, sodyum/potasyum bisülfit) kullanımıdır. Sülfitler antimikrobiyal ajanlar, indirgeyici ajanlar, beyazlatma ajanları ve antioksidan ajanlar olarak fonksiyon gösteren, hem enzimatik hem de enzimatik olmayan esmerleşmeyi kontrol edebilen maddelerdir. Buna rağmen, olumsuz sağlık etkilerine sebep olmasından dolayı Gıda ve İlaç Dairesi (FDA) tarafından ham madde olarak satılan ve direk tüketime sunulan meyve ve sebzelerde sülfitlerin kullanımı yasaklanmıştır. Bu sebeple enzimatik esmerleşme reaksiyonlarının kontrol altında tutulmasında kimyasal ajan olarak asitlendirici (sitrik asit, malik ve fosforik asit), indirgeyici (askorbik asit ve kombinasyonları) ve şelatlayıcı (EDTA) ajanlardan faydalanılmaktadır (Cerrahoğlu ve Arabacı, 2016). Bunun dışında mikrodalga ısıtma ve gamma ışınlaması gibi ışınlamaya dayalı yöntemler ile yüksek basınç, vurgulu elektrik alan, süperkritik karbondioksit ve ultrason uygulaması gibi ısıl olmayan yöntemler de enzimatik

esmerleşmeyi kontrol altında tutmak amacıyla kullanılan yeni teknolojilerdendir. Bu yeni teknolojilerin avantajlarının yanı sıra ekipman ve uygulama maliyetleri gibi önemli dezavantajları da bulunmaktadır. Bu teknolojilerin gıda sanayinde kullanımı maliyetlerinin dışında enzimler, mikroorganizmalar ve biyolojik dokular üzerindeki etkilerine de bağlı olarak değişiklik göstermektedir (Yılmaz ve Elmacı, 2018).

SONUÇ

Enzimatik esmerleşme reaksiyonları meyvelerin ve sebzelerin hasat, işleme ve depolama aşamalarında her yıl ciddi ekonomik kayıplara yol açmaktadır. PPO enzimi bu tip esmerleşmenin ana sebebidir ve ürünlerin sadece renginde kararmaya neden olmamakta, bunun yanı sıra lezzet ve kalitesinde de düşüşlere sebep olmaktadır. Bu sebeple gıda sanayinde PPO enzim inhibisyonu için en çok kullanılan yöntem, geleneksel suda haşlama metodudur. Bu yöntemin dışında buharlı haşlama, ohmik ısıtma, yüksek nemli sıcak hava haşlaması, mikrodalga ısıtma, gamma ışınlaması, yüksek basınç uygulaması, süperkritik karbondioksit uygulaması, vurgulu elektrik alan uygulaması ve ultrason uygulaması gibi yöntemlerden de faydalanılabilmektedir. Enzimatik esmerleşme karşıtı kimyasal ajanlar da, enzimatik esmerleşmeyi kontrol altında tutmak için kullanılan diğer bir yöntemdir. Kalitesi yüksek ürünler elde etmek için PPO enzim aktivitesini inhibe eden yöntemlerden bir ya da birkaçı kombine şekilde kullanılabilir.

KAYNAKÇA

Cerrahoğlu, E., & Arabacı, G. (2016). Eflatun çiçekli ballıbaba (lamium purpureum) polifenol oksidaz enziminin karakterizasyonu ve inhibisyonu. Sakarya University Journal of Science, 20(2), 245-250.

Dedeoğlu, N. (2009). Yenilebilir mantar türlerinden polifenol oksidaz enziminin saflaştırılması ve karakterizasyonu (Yüksek Lisans Tezi). Balıkesir Üniversitesi Fen Bilimleri Enstitüsü Kimya Anabilim Dalı, Balıkesir, 184s.

Ergün, A., & Çiçek, B. (2018). Bazı makrohalkalı tiyocrown eterlerin polifenol oksidaz enzimi üzerindeki inhibisyon etkilerinin araştırılması. Balıkesir Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 20(1), 539-546.

Gülsoy, E., Türkhan, A., & Kaya, E. D. (2019). Kimyasal ve elle meyve seyreltme uygulamalarının bazı elma çeşitlerinde polifenol oksidaz aktivitesi üzerine etkisi. Harran Tarım ve Gıda Bilimleri Dergisi, 23(2), 211-217.

Nakilcioğlu–Taş, E. (2016). Zeytin çekirdeğinin pilot ölçekte basınçlı su ekstraktörü kullanılarak su ekstraktının eldesi ve mikroenkapsülasyonu (Doktora Tezi). Ege Üniversitesi Fen Bilimleri Enstitüsü Gıda Mühendisliği Anabilim Dalı, İzmir, 242s.

Numanoğlu, E. N., & Çelik, A. G. (2018). Antioksidanların enzimatik esmerleşme üzerine etkileri. Bilim Armonisi, 1(1), 3-9.

Yılmaz, L., & Elmacı, Y. (2018). Polifenol oksidaz enzimi ve inaktivasyon yöntemleri. Türk Tarım-Gıda Bilim ve Teknoloji Dergisi, 6(3), 333-345.

BÖYÜK QAFQAZIN (AZƏRBAYCAN) ŞIMAL-ŞƏRQ HISSƏSINDƏ YAYILAN *CARPINUS* L.*VƏ CORYLUS* L. CINSLƏRINƏ AID NÖVLƏRIN EX SITU ŞƏRAITINDƏ MÖVSUMI INKIŞAFI (FENOLOGIYASI)

EX SITU SEASONAL DEVELOPMENT OF SPECIESOF *CARPINUS* L. AND *CORYLUS* L. GENUS DISTRIBUTED IN THE NORTH-EASTERN PART OF THE GREATER CAUCASUS (AZERBAIJAN)

Kəmalə Məmmədova Abdin qızı
Biologiya üzrə fəlsəfə doktoru, dosent
Azərbaycan Dövlət Pedaqoji Universiteti. Üzeyir Hacıbəyli 68. Bakı. Azərbaycan

ABSTRACT
An important role in evaluating the adaptation indicators of tree and shrub plants in new conditionsplaysthe study of the seasonal growth rhythm of the studied plants under cultural conditions.
Study the seasonal development rhythm of species belonging to the *Carpinus* L. and *Corylus* L. genus distributed in the north-eastern part of the Greater Caucasus (Azerbaijan) in *ex situ* conditions (Absheron)was the main goal of our research
The initiation of vegetation, the opening of shoots, and the end of vegetation were the shedding of leaves (up to 50%) of the studied tree and shrub plantsin the culture (Absheron) conditions.
Vegetation of *Corylus avellana L.* species started earlier in ex situ conditionswas determinedanalyzing the results of the phenological observations conducted on the studied. The vegetation in this species started with the opening of the flower buds (February).Leaves and flower buds opened in *Carpinus caucasica* Grossh. at the same time, in March. The duration of vegetation was 229 days in *Carpinus caucasica* Grossh., 228 in *C.orientalis*, and 266 in *Corylus avellana* L. was determinedduring the experiment.
Flowering in the studied species is earlier (in February) in *Corylus avellana,* for *Carpinus caucasica* and *C. orientalis* Mill., species in started in the middle period, in March-April., Flowering lasted for 18-19 daysdepending on the species
All 3 studied species bloom and bear fruit in the dry hebtropical climate of Absheroncan be notedaccording to the research results.
Key words: *ex situ, Carpinus, Corylus* phenology, vegetation, flowering.

Tədqiq edilən bitkilərin mədəni şəraitdə mövsümi inkişaf ritminin öyrənilməsi yeni şəraitdə ağac və kol bitkilərinin uyğunlaşma göstəricilərinin qiymətləndirilməsində mühüm rol oynayır.
Carpinus L. cinsi Cerepanova görə *Carpinaceae* Kuprian fəsiləsinə aid olub, Şimal yarım kürəsinin mülayim iqlim qurşağında yayılan 30-dan artıq növü təmsil edir (8,9).
Qafqazda və Azərbaycanda bu cinsin 6 növü, Böyük Qafqazın şimal-şərqində 2 növü (*Carpinus caucasica* Grossh., *C.orientalis* Mill.) yayılmışdır (7).
Corylus L. cinsi *Corylaceae* Mirb. fəsiləsinə aid olub, Avropanın və Asiyanın mülayim iqlim qurşağı meşələrində, Şərqi Asiyada və Şimali Amerikada 20 növü yayılıb (8,9). Qafqazda 6, Azərbaycanda 3 növü təbii meşələrdə bitir. Böyük Qafqazın şimal-şərqində isə 1 növü (*Corylus avellana* L.) meşə və kolluqlarda yayılmışdır (7). Qafqaz vələsi Azərbaycanda meşə əmələ gətirən növ olub, meşə sahəsinin 34%-ni təşkil edir. Bu meşələr tutduğu əraziyə görə fıstıq və palıq meşələrindən sonra 3-cü yeri tutur. Böyük Qafqazda vələs meşələri 67 min ha

sahə tutur, bu isə həmin ərazinin 17,5 %-ni təşkil edir. Vələs dəniz səviyyəsindən 1000-1500 m yüksəklikdə daha geniş yayılmışdır. Bu növün Böyük Qafqazın orta və yuxarı dağ qurşağında rütubətli iqlim şəraitində meşəliklər əmələ gətirməsi, meşənin yuxarı sərhədinə kimi qalxması, Samur-Şabran düzənliyində dəniz səviyyəsinədək enməsi vələs ağaclarının geniş iqlim amplitudu şəraitində yayılmasını təsdiq edir (3).

Qafqaz vələs mülayim rütubətli gillicəli torpaqlarda daha geniş yayılır (6).

Axundova görə qafqaz vələsi növünün 3 növmüxtəlifliyi vardır. Bu növ müxtəlifliklərindən biri kölgəli və rütubətli bitmə şəraitində, digəri daha quru bitmə şəraitində, üçüncüsü isə açıqlıqda, daşlı sahələrdə rast gəlir (4).

Şərq vələsi Böyük Qafqazın şimal-şərq hissəsində Quba, Qusaq, Şabran rayonlarında aşağı və orta dağ qurşaqlarında palıd və vələs meşələrinin kolluqlarının tərkibində bitir (1,2).

Adi fındıq Böyük Qafqazın şimal-şərq hissəsində Samur-Şabran düzənliyindən başlamış orta dağ meşə qurşağında meşələrin tərkibində və kolluqlarda bitir. Quba, Qusar və Şabran rayonları ərazisində geniş sahələrdə fındıq koluqları əmələ gətirir.

Böyük əhəmiyyət kəsb edən bu ağac və kol bitkilərinin ex situ şəraitində (Abşeron) mövsümi inkişaf ritminin öyrənilməsinin qarşımıza məqsəd qoymuşduq.

Material və metodika

Tədqiqat Abşeron şəraitində AMEA-nın Mərkəzi Nəbatat bağında aparılmışdır. Bağda introduksiya edilən 3 növ (*Carpinus caucasica* Grossh., *C.orientalis Mill., Corylus avellana* L.) üzərində fenoloji müşahidələr aparılmış və bu növlərin ex situ şəraitində mövsumi inkişaf ritmi öyrənilmişdir. Tədqiqatda Beydmanın metodikasından istifadə edilmişdir (5).

Nəticələr və onların müzakirəsi

Ex situ şəraitində tədqiq olunan növlərin mövsümi inkişaf ritmini öyrənmək üçün fevral ayından başlayaraq bu növlər üzərində fenoloji müşahidələr aparılmışdır. Öyrənilən ağac və kol bitkilərinin mədəni şəraitdə (Abşeron) vegetasiyasının başlanması tumurcuqların açılması vegetasiyanın qurtarması isə yarpaqların tökülməsi ilə (50%-ə qədər) qəbul edilmişdir.

Abşeron şəraitində tədqiq edilən növlər üzərində aparılan fenoloji təcrübələrin nəticələrini təhlil edərkən müəyyən olunmuşdur ki, vegetasiyanı daha tez başlayan Corylus avellana növü olmuşdur. Bu növdə vegetasiya çiçək tumurcuqlarının açılması ilə (27.02) başlamışdır. Carpinus caucasica Grossh. növündə yarpaq və çiçək tumurcuqları eyni vaxtda mart ayında (24.03), C. orientalis növündə aprel ayında (2.04) açılmışdır. Corylus avellana növündə yarpaq tumurcuqları aprel ayında açılmışdır (2.04).

Bu növlərdə tam yarpaqlama aprelin 27-28-ci arasında müşahidə edilmişdir (cədvəl 1.)

Carpinus və Corylus cinsinin nümayəndələrində çiçək bircinsli, birevlidir. Vələs cinsinin erkək çiçəkləri sırğaya, dişi çiçəkləri 2 çiçəkli dixazilərdən ibarət sırğaya toplanmışdır. Fındıqda erkəkcikli çiçək qrupu sadə sırğalardır. Dişi çiçəkləri isə birillik zoğun ucunda yerləşmiş dixazilərdən ibarətdir. Erkəkcikli sırğaları 2-5 sm olub, 3-5 ədədi bir yerə toplanır. Belə sırğalar payızda əmələ gəlir və bütün qışı ağacda qalır.

Tədqiq olunan növlərdə çiçəkləmə daha tez (fevral ayında) *Corylus* avellana növündə Qafqaz və Şərq vələsi növlərində orta müddətdə mart, aprel aylarında başlanmışdır. Növlərdən asılı olaraq çiçəkləmə 18-19 gün davam etmişdir. Meyvə isə çiçəkləmədən 13-15 gün sonra başlanmışdır. Meyvənin yetişməsi avqust-sentyabr aylarında qeydə alınmışdır (şəkil 1, 2, 3, 4).

Şəkil 1. *Corylus avellana*-da erkək-cikli sırğalar.

Şəkil 2. *Corylus avellana*-nın meyvəli budağı

Şəkil 3. *Carpinus caucasica*-nın meyvəli budağı

Şəkil 4. *Carpinus orientalis*-in meyvəli budağı

Öyrənilən növlərdə yarpaqların tökülməsi noyabrın II öngünlüyündə müşahidə edilmişdir. Aparılan tədqiqat zamanı ex situ şətaitində vegetasiyanın davametmə müddətinin *Carpinus caucasica* növündə 229 gün, *C.orientalis*-də 228 gün, *Corylus avellana* növündə 261 gün olduğu müəyyən edilmişdir.

Tədqiqatın nəticələrinə görə qeyd etmək olar ki, öyrənilən hər 3 növ Abşeronun quru subtropik iqlim şəraitində çiçəkləyir və meyvə verir.

Cədvəl 1.

Carpinus* L. *və Corylis* L. *cinsinə aid növlərin ex situ (Abşeron) şəraitində mövsümi inkişafı (fenologiyası)

№	Növ	Tumur-cuqların açılması	Tam yarpaq-lama	Çiçəkləmə		Çiçək-ləmə müd-dəti	Mey-vənin tam yetiş-məsi	Yarpaq-ların saral-ması	Yar-paqla-rın tö-kül-məsi	Vege-tasiya-nın davam etmə müddəti
				Başlan-ması	Sonu					
1	*Carpinus caucasica*	24.03	17.04	25.03	13.04	18	25.09	15.09	8.11	229
2	*C. orientalis*	2.04	28.04	4.04	4.04	19	6.10	21.09	16.11	228
3	*Corylus avellana*	27.02	27.04	28.02	28.02	16	25.08	11.09	15.11	261

Ədəbiyyat

1. Əsədov K.S., Məmmədov F.M., və b. Böyük Qafqazın Şimal-Şərq hissəsinin dendroflorası və meşələri. Bakı. Bakı Universiteti, 2008, 276 s.

2. Əsədov K.S., Mirzəyev O.H., Məmmədov F.M.. Dekdrologiya, Bakı, Gənclik, 2014, 484 s.

3. Məmmədov Q., Xəlilov M. Azərbaycanın meşələri, Bakı, Elm, 2002, 472 s.

4. Ахундов Н.Г. Горное лесоразведение в Азербайджкой ССР и перспективы его развития: Автореф. канд. дисс. Баку, 1963, 30 с.

5. Бейдеман И.Н. Методика изучения фенологии растений и растительных сообществ. Новосибирск: Наука, 1999, 185 с.

6. Прилипко Л.И. Лесная растительность Азербайджана. Баку, Изд. АН Азерб. ССР, 1954. 485 с.

7. Флора Азербайджана Т.3 Баку: АН Азерб.ССР, 1952, 407 с.

8. Черепанов С.К. Сосудистые растения СССР, Ленинград, «Наука», 1981, 510 с.

9. Черепанов С.К. Сосудистые растения России и сопредельных государств (в пределах бывшего СССР) Санкт-Петербург 1995, 990 с.

AZƏRBAYCANIN DAĞ EKOSİSTEMİ XÜSUSİ QORUNAN ƏRAZİLƏRİN NÜMUNƏSİNDƏ

Mahmudov Yusif Məhəmməd oğlu
Ekoloq, tarix üzrə fəlsəfə doktoru
Azərbaycan Respublikası

XÜLASƏ

Ətraf mühitə qayğıkeş münasibət təbii komplekslərin qorunub saxlanmasında vacib məsələlərdən biridir. Aktuallıq kəsb edən ekoloji problemlərin həlli istiqamətində atılan hər bir addım, görülən real tədbirlər təbiətin xeyrinədir. Müasir Azərbaycan Respublikasında ətraf mühitə diqqət göstərilməsi təccüblü deyildir. Ölkənin sosial-iqtisadi inkişafı və onun potensial imkanları bu sahədə daha çox işlər görməyə şərait yaradır. Sovet hakimiyyəti illərində də ekoloji problemə az-çox diqqət yetirilib, lakin qəbul edilən qanun və qərarlar, həyata keçirilən tədbirlər ciddi səmərə verməyib. İnzibati idarəetmə üsulu ekoloji tədbirlərin həyata keçirilməsinə mane olub və ya onu ləngidib. 1991-ci ildə yenidən bərqərar olan dövlət müstəqilliyi dönəmində Azərbaycanda ətraf mühit problemlərinə beynəlxalq standartlar çərçivəsində və daha ciddi yanaşılıb.

Tədqiqatlar göstərir ki, 2001-ci ildə yaranan Ekologiya və Təbii Sərvətlər Nazirliyi mövcud problemin həlli istiqamətində kəskin addımlar atılıb. Dövlət əhəmiyyətli qoruq və yasaqlıqların ərazisi genişləndirilib. Məqalədə həmçinin qeyd olunur ki, müstəqillik dövründə daha bir neçə qoruq və yasaqlıq, 2003-cü ildən sonra isə milli parklar təşkil olunub. Bəzi qoruq və yasaqlıq-ların əraziləri birləşdirilərk, onların əsasında yeni qoruq və milli parklar yaradılıb. Hazırda ölkədə 24 yasaqlıq, 10 qoruq və 10 milli park vardır.

Qeyd etmək lazımdır ki, xüsusi qorunan ərazilərin – yasaqlıq, qoruq sahələri və ekoparkların bir çoxu dağlıq ərazilərdə yaradılmışdır [http://eco.gov.az]. Qoruqlardan: Zaqatala DTQ (1929), Türyançay DTQ (1958), İlisu DTQ (1987), Qaragöl DTQ (1987), Korçay DTQ (2008); yasaqlıqlardan: Laçın DTY (1961), Qusar DTY (1964), İsmayıllı DTY (1969), Zuvand DTY (1969), Qubadlı DTY (1969), Korçay DTY (1961), Ordubad DTY (1969), Daşaltı DTY (1981), Qızılca DTY (1984), Zaqatala DTY (2008) və s.; milli parklardan: Altıağac MP (2004), Hirkan MP (2004), Şahdağ MP (2006) və Göygöl MP (2008). Bu cür qorunan ərazilərdə həm flora və fauna, həm də cansız təbiət nümunələri bir-birləri ilə qarşılıqlı əlaqə şəklində mövcuddurlar.

Azərbaycanda ekoturizm üçün faydalı olan və dünya standartlarına cavab verən, 2016-cı ildə təşkil olunmuş Safari Parkı Şamaxı rayonunda Pirqulunun dağ-meşə massivində yerləşir. Ərazidə Latviya, Polşa, Slovakiya, Çexiya və Macarıstan təsərrüfatlarından gətirlərək parka buraxılan üç növdən ibarət nəcib maral, lan və muflon adlı genetik qiymətli heyvanlar xüsusi mühafizə altındadır.

Bu cür xüsusi rejimli qorunan ərazilər bir çox cəhətdən əhəmiyyətli olmuşdur. Nadir və nəsli kəsilməkdə olan növlərin öz təbii yaşayış yerlərində mühafizə edilib saxlanması onların sayca artması ilə nəticələnib. Beləliklə, biomüxtəlifliyin saxlanması və burada ekoturizmin inkişafı üçün real imkanlar yaranıb.

Açar sözlər: dağ ekosistemi, ətraf mühit, ekoloji problem, qoruq və yasaqlıq, milli park, ekopark, ekoloji tədbirlər, biomüxtəliflik.

AZERBAYCAN'IN DAĞ EKOSİSTEMİ ÖZEL KORUMA ALANLARINA BİR ÖRNEK OLARAK

ÖZET

Çevreye özen göstermek, doğal komplekslerin korunmasında önemli konulardan biridir. Acil ekolojik sorunların çözülmesi yönünde atılan her adım ve alınan gerçek önlemler doğanın yararınadır. Modern Azerbaycan Cumhuriyeti'nde çevreye dikkat edilmesi şaşırtıcı değildir. Ülkenin sosyo-ekonomik gelişimi ve sahip olduğu potansiyel fırsatlar bu alanda daha fazla çalışma yapılması için koşullar yaratmaktadır. Sovyet yönetimi yıllarında bile, ekolojik soruna az çok dikkat edilmiş, ancak kabul edilen yasa ve kararlar ile uygulanan önlemlerin ciddi bir etkisi olmamıştır. İdari yönetim yöntemi, çevresel önlemlerin uygulanmasını engelledi veya geciktirdi. 1991 yılında yeniden kurulan devlet bağımsızlığı döneminde, Azerbaycan'daki çevre sorunları uluslararası standartlar çerçevesinde daha ciddi bir şekilde ele alındı.

Yapılan araştırmalar, 2001 yılında kurulan Ekoloji ve Tabii Kaynaklar Bakanlığı'nın mevcut sorunu çözmek için ciddi adımlar attığını gösteriyor. Ulusal olarak önemli rezervler ve koruma alanları genişletildi. Makale ayrıca, bağımsızlık döneminde birkaç doğa koruma alanı ve kutsal alanın ve 2003'ten sonra milli parkların kurulduğundan bahseder. Bazı doğa rezervlerinin ve kutsal alanların toprakları birleştirildi ve bunlara dayalı olarak yeni doğa rezervleri ve milli parklar oluşturuldu. Şu anda ülkede 24 kutsal alan, 10 doğa rezervi ve 10 milli park bulunmaktadır.

Bu koruma alanlarının ve ekoparkların birçoğunun dağlık alanlarda oluşturulduğuna dikkat edilmelidir. Rezervlerden: Zagatala DDR (1929), Turyançay DDR (1958), Pirqulu DDR (1968), İlisu DDR (1987), Karagöl DDR (1987), Korçay DDR (2008); koruma alanlarından: Lachin DDKA (1961), Korchay DDKA (1961), Gusar DDKA (1964), Ismayilli DDKA (1969), Gubadlı DDKA (1969), Zuvand DDKA (1969), Ordubad DDKA (1969), Dashalti DDKA (1981), Kızılca DDKA (1984), Zagatala DDKA (2008) vb.; milli parklardan: Altyağaç MP (2004), Hirkan MP (2004), Şahdağ MP (2006) ve Göygöl MP (2008). Bu tür korunan alanlarda hem flora ve fauna hem de cansız doğa birbirleriyle etkileşim halinde bulunmaktadır.

Azerbaycan'da ekoturizm için faydalı olan ve 2016 yılında düzenlenen dünya standartlarını karşılayan Safari Parkı, Shamakhi ilçesinde Pirgulu dağ-orman masifinde yer almaktadır. Letonya, Polonya, Slovakya, Çek Cumhuriyeti ve Macaristan'daki çiftliklerden getirilen kızıl geyik, vaşak ve yaban koyunu olmak üzere üç türden genetik olarak değerli hayvanlar özel koruma altındadır.

Bu tür özel rejim koruma alanları birçok yönden önemli olmuştur. Nadir ve nesli tükenmekte olan türlerin doğal ortamlarında korunması, sayılarının artmasına neden olmuştur. Bu nedenle, burada biyolojik çeşitliliğin korunması ve ekoturizmin geliştirilmesi için gerçek fırsatlar var.

Anahtar Kelimeler: dağ ekosistemi, çevre, ekolojik sorun, rezerv ve koruma alanı, milli park, ekopark, çevresel önlemler, biyolojik çeşitlilik.

AZERBAİJAN'S MOUNTAİN ECOSYSTEM AS AN EXAMPLE OF SPECİAL PROTECTED AREAS

RESUME

Taking care of the environment is one of the important issues in the protection of natural complexes. Every step taken and real measures taken towards solving urgent ecological problems is for the benefit of nature. It is not surprising that in the modern Republic of Azerbaijan, attention is paid to the environment. The socio-economic development of the country and its potential opportunities create conditions for further work in this area. Even during the years of Soviet rule, more or less attention was paid to the ecological problem, but the laws and resolutions adopted and the measures implemented did not have a serious impact. The administrative method of management prevented or delayed the implementation of environmental measures. During the period of state independence, which was re-established in 1991, environmental problems in Azerbaijan were handled more seriously within the framework of international standards.

Research shows that the Ministry of Ecology and Natural Resources, established in 2001, has taken serious steps to solve the current problem. The territory of nationally important reserves and sanctuaries was expanded. The article also mentions that several more reserves and sanctuaries were created during the independence period. The article also mentions the establishment of several nature reserves and sanctuaries during independence and national parks after 2003. The territories of some nature reserves and sanctuaries were combined and new nature reserves and national parks were created based on them. Currently, there are 24 sacred areas, 10 nature reserves and 10 national parks in the country.

It should be noted that many of these protected areas and ecoparks were created in mountainous areas. From the reserves: Zagatala SNR (1929), Turyanchay SNR (1958), Pirgulu SNR (1968), İlisu SNR (1987), Garagol SNR (1987), Korchay SNR (2008); from protection areas: Lachin SNPA (1961), Gusar SNPA (1964), Korchay SNPA (1961), Ismayilli SNPA (1969), Zuvand SNPA (1969), Gubadli SNPA (1969), Ordubad SNPA (1969), Dashalti SNPA (1981), Kyzylca SNPA (1984), Zagatala SNPA (2008), etc.; from national parks: Altyagac NP (2004) and Hirkan NP (2004), Shahdag NP (2006), and Goygol NP (2008). In such protected areas, both flora and fauna and inanimate nature interact with each other.

The Safari Park, which is beneficial for ecotourism in Azerbaijan and meets the world standards organized in 2016, is located in the Pirgulu mountain-forest massif in Shamakhi district. Genetically valuable animals from three species: red deer, lynx and wild sheep brought from farms in Latvia, Poland, Slovakia, the Czech Republic and Hungary are under special protection.

Such special regime protection areas have been important in many ways. Conservation of rare and endangered species in their natural habitat has resulted in an increase in their numbers. Therefore, there are real opportunities here for the conservation of biodiversity and the development of ecotourism.

Keywords: mountain ecosystem, environment, ecological problem, reserve and protection area, national park, ecopark, environmental measures, biodiversity.

Giriş.

Azərbaycanda əhalinin ətraf mühitə qayğıkeş münasibətinin tarixi çox qədim dövrlərə gedib çıxır. Ancaq onun dövlət səviyyəsində mühafizəsinə ötən əsrin əvvəllərindən başlamışdır və bu gün də uğurla davam etdirilir. Bu sahədə görülən tədbirlər öz səmərəsini verməkdədir. Qorunan ərazilərdə nadir və nəsli kəsilmək üzrə olan boloji növlərin sayca artması

tendensiyası özünü göstərir. Bu baxımdan xüsusi rejimlə qorunan ərazilərin təşkili təbiətin keyfiyyət göstərici-lərinin saxlanılmasında əsas uğurlardan hesab oluna bilər.

Qeyd etmək lazımdır ki, ölkədə ətraf mühitin qorunması ilə bağlı ilk tədbirlər Azərbaycan Xalq Cumhuriyyəti dövründə həyata keçirilib **[**Azərbaycan Xalq Cumhuriyyəti Ensiklopediyası. I c.: 2004**].** Azərbaycan SSR-də bu proses daha da inkişaf etdirilib və dövlət siyasəti səviyyəsinə qaldırılıb. Relyefinin müxtəlifliyinə baxmayaraq, ölkəmizin ərazilərində çoxlu sayda qoruq və yasaqlıqlar, milli parklar yaradılıb. Son illərdə yaradılan Safari Parkı dağlıq yerlərdə yaşamağa uyğunlaşmış bəzi vəhşi heyvan növlərini qorunması üçün nəzərdə tutulub. Bu cür xüsusi qorunan ərazilərin yaradılması həm ekoturizmin inkişaf etdirilməsi, həm də dağ ekosisteminin formalaşması üçün müstəsna əhəmiyyət kəsb edir.

Məlum olduğu kimi Qarabağ və ona bitişik rayonların 20 faizdən çox ərazisi təcavüzkar Ermənistan Ordusu tərəfindən zəbt olunmuş və Azərbaycan Respublikasının 2 dövlət qoruğu, 2 dövlət yasaqlığı, çoxlu təbiət abidələri, o cümlədən çoxyaşlı ağaclar, meşəliklər və kolluqlar, digər təbii srvətlər 30 ilədək işğal atında qalmışdır [Işğal olunmuş Azərbaycan ərazilərində ətraf mühitə və təbii sərvətlərə dağıdıcı təsiri müəyyənləşdirən (izləyən) operativ mərkəzin I hesabatı: 2002]. 2020-ci il oktyabrın 25-də Qubadlı, noyabrın 8-də Şuşa, dekabrın 1-də Laçın rayonu işğaldan azad edilmişdir. İşğal dövründə erməni işğalçıları tərəfindən ərazinin təbii sərvətlərinə və təbii mühitinə ciddi ziyan vurulmuş, meşələr qırılmış, təbii ekosistemləri məhv edilmiş,, o cümlədən dağ ekosistemləri dağıdılmış, vəhşi heyvanlar öz yaşayış areallarını tamamilə itirmişlər [Mahmudov, 2022: s.80-121].

Hazırda işğaldan azad olunmiş ərazilərdə bioloji müxtəlifliyin mövcud vəziyyətinin öyrənilməsi məqsədilə Ekologiya və Təbii Sərvətlər Nazirliyində yaradılmış İşçi qrupun fəaliyyəti çərçivəsində həmin ərazilərdə fauna və flora aləminin növ tərkibinin yenidən öyrənilməsi və təbiət abidələrinin invertarlaşdırılması istiqamətində monitorinq işləri aparılır [http://eco.gov.az].

Material və metodlar.

Məqalənin materialını müəllifin apardığı tədqiqatlar, elmi ədəbiyyatlar, rəsmi ekoloji nəşrlər, internet resursları, Azərbaycan Respublikasının ekoloji qanunvericiliyi, kollektiv monoqrafiyalar və s. təşkil edir.

Tədqiqat prosesində müqayisə və çoxparametrli araşdırma üsulundan istifadə olunmuşdur.

Aparılan müakirələr və alınan nəticələr.

Hirkan MP - 2004-cu ildə 21435 hektar ərazidə yaradılıb. Sonradan parkın ərazisi 18923 ha genişləndirilərək 40358 ha çatdırılıb. Milli Park (MP) Azərbaycanın cənubi-şərqində, Lənkəran və Astara inzibati rayonlarının ərazisində yerləşir və Talış dağlarının unikal təbiət komplekslərı ilə fərqlənən təbii ərazilərini əhatə edir. MP-nın yaradılmasında əsas məqsəd həmin ərazidə təbiətin kompleks qorunması, Üçüncü dovrun relikt və endemik bitki növlərinin muhafizəsi, Azərbaycan Respublikasının Qırmızı kitabına daxil edilmiş tipik flora və fauna növlərinin qorunub saxlanılması, ətraf mühitin monitorinqinin həyata kecirilməsi, ictimaiyyətin məlumatlandırılması, elmi tədqiqatlar, turizm və istirahət ücün şərait yaratmaqdır. MP-nın meşələrində ayıdöşəyi (*Dryopteris*), Hirkan bigəvəri (*Ruscus Hyrcanus*), Hirkan şümşadı (*Buxus hyrcana*), pırkal (*Ilex*), muxtəlif lianlar (*Liana*) bitir. Hirkan MP-nın yerləşdiyi Lənkəran bölgəsinin subtropik meşələrinin səciyyəvi xususiyyətlərindən biri də burada Hirkan tipli meşələrin yaxşı saxlanılması, bir cox endemik, nadir ağac və kol bitkilərinin yayılmasıdır. Hirkan MP-nın ərazisində əsasən İpək akasiya (*Albizzia julibrissin*), Xəzər lələyi (*Gleditsia caspica*), Hirkan ənciri (*Ficus hyrcana*), Qafqaz xurması (*Diospyros lotus*), budaqlı danaya (*Danae racemosa*), Hirkan bigəvəri (*Ruscus hyrcanus*), Pastuxod daşsarmaşığı (*Hedera pastuchovii*), Buasye armudu (*Pyrus boissieriana*), qanadmeyvə yalanqoz (*Pterocarya pterocarpa*), ürəkyarpaq qızılağac (*Alnus subcordata*), dəmirağac (*Parrotia persica*), şabalıdyarpaq palıd (*Quercus castaneifolia*), vələsyarpaq azatağacı (*Zelkova carpinifolia*), vələs (*Carpinus orientalis*), şərq fıstığı (*Fagus orientalis*) və s.

qorunur. MP-nın entomofaunası endem taksonlarla (növ, cins, fəsilə) zəngindir. Burada Qırmızı kitaba daxil edilən 15-dən çox kəpənək növləri burada qorunub saxlanır. Quşlardan qara leylək (*Ciconia nigra*), hirkan arıquşu (*Parus hyrcanus*), qaratoyuq (*Turdus torquata*), sığırçın (*Sturnus vulgaris*), çöl muymulu (*Falco naumanni*), Talış qırqovulu (*Phasianus colchicus talischensis*), Türküstan qırğısı (*Accipiter badius*), məməlilərdən çöl donuzu (*Sus scrofa*), porsuq (*Meles meles*), yenot (*Procyon lotor*), canavar (*Canis)*, tülkü (*Vulpes vulpes*), bəbir (*Panthera pardus*), xallı maral (*Cervus nippon*), vaşaq (*Lynx*), əlik cüyür (*Capreolus*) və s. növlər yaşayır.

və s. olur. Suda-quruda yaşayan heyvan növlərindən adi triton, daraqlı triton, Suriya sarımsaqiyli qurbağası, Qafqaz xaclıcası, adi quru qurbağası yayılıb. Sürünənlərdən Aralıqdəniz tısbağası, zolaqlı çılpaqgoz (kərtənkələ), yovşanlıq girdəbaşı, xarabalıq kələzi, Eskulap sarmaşantəlxəsi və s. növlər yayılıb. Hirkan MP-nın ornitofaunasına daxil olan qara leylək (*Ciconia nigra*), məzar qartalı (*Aquila heliaca)*, Talış qırqovulu (*Phasianus colchicus talischensis*), Hirkan arıquşu (*Poecile hyrcanus*) və s. quşların adları Azərbaycanın Qırmızı kitabına daxil edilib. Parkın ərazisində Qırmızı kitaba daxil olan növlərdən Hirkan şümşəsi (*Ilex hyrcana*), Hirkan qovağı (*Populus hyrcana*), Lənkəran gülxətmisi (*Alcea lenkoranica*), giləmeyvəli qaraçöhrə (*Taxus baccata*), Talış uzunbığı (*Purpuricenus talyshensis*), Talış məxməri kəpənəyi (*Lasiommata adrastoides*), qara leylək (*Ciconia nigra*), cırtdan qartal (*Hieraaetus pennatus*), tirəndaz (*Hystrix indica*), Ön Asiya bəbiri (*Panthera pardus saxicolor*), qonur ayı (*Ursus arctos*), vaşaq (*Lynx lynx*), zolaqlı kaftar (*Hyaena hyaena*) və s. Hirkan MP-da 100-dən cox təbiət və mədəniyyət abidələri var [Mustafayev, Mahmudov: 2016, s.95-110; http://eco.gov.az/az/77-milli-parklar; http://eco.gov.az/index.php?ln=az&pg=83].

Altıağac MP - 2004-cu ildə Xızı və Siyəzən inzibati rayonlarının ərazilərində, Altıağac DTQ və ona həmsərhəd olan dövlət meşə fondu torpaqlarının bazasında yaradılıb. Ərazisi 11035 hektardır. Bu Parkın yaradılmasında əsas məqsəd Boyuk Qafqazın cənub-şərq yamaclarının təbii landşaftlarının, flora və faunasının qorunub saxlanılması, bərpası, təbii komplekslərin turizm və istirahət (rekreasiya) üçün şəraitin yaradılması və əhalinin ekoloji cəhətdən maarifləndirilməsinə xidmətdir. Ərazisinin cox hissəsini meşələr əhatə edir. Buradakı meşələri əmələ gətirən əsas ağac növlərindən Qafqaz vələsi (Carpinus caucasica), adi göyrüş (Fraxinus excelsior), Şərq fıstığı (*Fagus orientalis*), əzgil (*Mespilus germanica*), zirinc (*Berberis vulgaris*), yemişan (*Crataegus pentagyna*), Qafqaz palıdı *(Quercus)*, itiyarpaqlı ağcaqayındır *(Aser L.)*. Parkın ərazisində böyurtkən *(Eubatus)*, əzgil *(Mespilus)*, qaratikan *(Paliusrus)*, itburnu *(Rosa) və s.* kimi kol bitkiləri də geniş yayılıb. Cavan meşələr çoxluq təşkil edir. Oreofil və petrofil növlər çoxalıb.

Milli Parkda məməlilərdən əlik cüyur, qonur ayı, vaşaq pişik, boz canavar (*Canis lupus*), çöl donuzu (Sus scrofa), bildirçin (*Coturnix coturnix*), Amerika yenotu (*Procyon lotor*), boz dovşan (*Lepus europaeus*), tülkü (*Vulpes vulpes*), meşə dələsi (*Martes mastes*); quşlardan bildirçin, cobanaldadan, qartal, qaratoyuq (*Turdus torquatus*), alabaxta (*Columba palumbus*), kəklik (*Alectoris chukar*) və başqa növlər yaşayır. Ərazidə Azərbaycanın Qırmızı kitabına daxil edilmiş qartal növləri diqqəti daha cox cəlb edir. Bundan başqa Qonur ayı (*Ursus arctos*), vaşaq (*Lynx lynx*), Avropa cüyürü (*Capreolus capreolus*), saqqallı quzugötürən (*Gypaetus barbatus*), qara çalağan (Milvus migrans), kəskiniyli ardıc (*Juniperus foetidissima*), Qafqaz bənövşəsi (*Viola caucasica*), itikənarlı süsən (*Iris acutiloba*) və s. vardır. MP-nın uğurları gələcəyə ümidi çoxaldır [Mustafayev, Mahmudov: 2016, s.95-110; http://eco.gov.az/az/77-milli-parklar; http://eco.gov.-az/index.php?ln=az&pg=83].

Şahdağ MP - 2006-cı ildə yaradılıb. Ərazisi ilkin olaraq 115895 hektar təşkil edir ki, bundan İsmayıllı və Pirqulu DTQ-ları 21014 ha, Quba, Qusar, İsmayıllı, Qəbələ, Oğuz və Şamaxı inzibati rayonlarının ərazilərində olan dövlət meşə torpaqları 81797 ha və həmin rayonların hüdudlarındakı yuksəkliklərdə yerləşən yaylaqlar isə 13084 ha sahəni əhatə edir.

Şahdağ MP-nın yaradılmasında məqsəd endemik və nəsli kəsilməkdə olan novlər və transsərhəd miqrant heyvanlar da daxil olmaqla qlobal əhəmiyyətli ekosisteminin bərpası, stabilliyinin qorunması və idarə edilməsidir. MP-ın relyefinin şaquli qurşaqları burada bitki örtüyünün çox muxtəlifliyinə səbəb olub. Meşələr, əsasən iberiya, Şərq palıdı (*Quercus macranthera*), Qafqaz vələsi (*Carpinus caucasica*), Şərq fıstığı (*Fagus orientalis*), göyrüş (*Fraxinus excelsior*), qaraçöhrə (*Taxus baccata*), ağcaqayın (*Acer campestre*), söyüd, yunan qozu (*Juglans regia*), gilas, alma (*Malus orientalis*), armud, əzgil (*Mespilus germanica*), yemişan (*Crataegus* sp.), böyürtkan (*Rubus buschii*), itburnu (*Rosa canina*), zirinc (*Berberis vulgaris*) və s.-dən ibarətdir. MP-nin cox boyuk ərazini əhatə etməsi nəticəsində burada monotərkib, həm də qarışıq meşələr var: Göyrüş (*Fraxinus excelsior*), Şərq palıdı (*Quercus macranthera*), Qafqaz vələsi (*Carpinus caucasica*), Şərq fıstığı (*Fagus orientalis*), qaraçöhrə (*Taxus baccata*), ağcaqayın (*Acer campestre*), söyüd, yunan qozu (*Juglans regia*), gilas, alma (*Malus orientalis*), armud, əzgil (*Mespilus germanica*), yemişan (*Crataegus* sp.) və başqa ağaclar, dəmirqara, əzgil, yemişan, böyürtkan (*Rubus buschii*), itburnu (*Rosa canina*), zirinc (*Berberis vulgaris*) və s. meşələr.

Şahdağ MP-nın faunası zəngindir: Quşlardan qırqovul (Phasianus), bildircin (*Coturnix coturnix*), qaratoyuq (*Turdus*), alabaxta (*Columba palumbus*), sarıköynək (), çobanaldadan (), ağacdələn (*Picinae*), məməlilərdən Şərqi Qafqaz turu (*Capra cylindricornis*), dələ (*Martes*), əlik cüyür (*Capreolus*), maral (*Cervidae*), qarapaca (*Rupicapra rupicapra*), çöl donuzu (*Sus scrofa*), qonur ayı (*Ursus arctos*), canavar (*Canis*), çaqqal (*Canis aureus*), tülkü (*Vulpes*), yenot (*Procyon*), meşə pişiyi (*Felis silvestris silvestris*), vaşaq (*Lynx*), gəlincik (*Mustela*), porsuq (*Meles meles*), dovşan (*Lepus*) və s. yayılıb.

MP-da Azərbaycan Respublikasının Qırmızı kitabına daxil edilmiş növlərdən Qonur ayını (*Ursus arctos*), nəcib maralı (*Cervus elaphus*), Avropa cüyürünü (*Capreolus capreolus*), Qafqaz köpgərini (*Rupicapra rupicapra*), vaşaqı (*Lynx*), bərqud qartalnı (*Aquila chrysaetos*), imperator qartalını, Qafqaz tetrasını (*Lyrurus mlokosiewiczi*), Qafqaz ularını (*Tetraogallus caucasicus*) və b.-nı göstərmək olar. [Mustafayev, Mahmudov: 2016, s.95-110; http://eco.gov.az/az/77-milli-parklar; http://eco.gov.az/index.php?ln=az&pg=83].

Göygöl MP - 2008-ci ildə Göygöl, Goranboy və Daşkəsən inzibati rayonlarının 12755 hektar sahəsində, Göygöl DTQ-nun bazasında yaradılıb. Parkın əsas obyektləri Kiçik Qafqazın şimal yamaclarının subalp zonasının bir hissəsi, orta dağlıq meşələri, Göygöl, Maralgöl və Zəligöl ekosistemləridir. Göygöl MP-da dağ-meşə, dağ-bozqır, subalp və alp yüksək dağ-çəmən qurşaqları böyük və ümumi ekosistem yaradır. Göygöl Milli Parkı zəngin bitki örtüyünə malikdir. 1100-2200 m hündürlükləri əhatə edən dağ meşələri 80-ə qədər ağac və kol növünə malikdir. Meşələrin əsas hissəsini kiçik yarpaqlı ağaclardan şərq fıstığı (*Fagus orientalis*), şərq palıdı (*Quercus macranthera*), Qafqaz vələsi (*Carpinus caucasica*), tozağacı (*Betula pendula*), adi göyrüş (*Fraxinus excelsior*), ağcaqayın (*Acer campestre*), cökə (*Tilia cordata*), iynəyarpaqlı ağaclardan isə qarmaqvari və Kox şamı (*Pinus kochiana*) təşkil edir. Kol bitkilərindən zoğal, zirinc, itburnu, əzgil, adi gərməşov, böyürtkən və s. yayılmışdır. Ot bitkilərindən Qafqaz bənövşəsi (*Viola caucasica*), alp dişəsi (*Poa alpina*), Steven zəncirotu (*Taraxacum stevenii*), zəngçiçəyi (*Campanula* sp.), adi çobanyastığı (*Anthemis* sp), şırımlı topal (*Festuca rupicola*), uzanan ağbığ (*Nardus stricta*), adi qırtıc (*Poa bulbosa*) və s. göstərmək olar. Bu növlər ən çox subalp çəmənliyində yayılmışdır. Milli parkın ərazisinin 2000-ə qədər hündürlüyü əhatə etməsi, müxtəlif bitki formasiyalarının bir-birinə əvəz etməsi, ərazinin ümumi coğrafi mövqeyi burada heyvanlar aləminin də xeyli zənginliyinə şərait yaratmışdır. Göygöl Milli Parkının ərazisində məməli heyvan növlərindən nəcib və ya Qafqaz maralı (*Cervus elaphus*), Avropa cüyürü (*Capreolus capreolus*), qonur ayı (*Ursus arctos*), dağ keçisi (*Capra aegagrus*), porsuq (*Meles meles*), meşə pişiyi (*Felis silvestris*), vaşaq (*Lynx lynx*), daşlıq dələsi (*Martes foina*), gəlincik (*Mustela erminea*), adi kirpi (*Erinaceus concolor*), tülkü (*Vulpes vulpes*), Radde qonurdişi (*Sorex raddei*), xırda köstəbək (*Talpa levantis*),

ağqarın ağdiş (*Crocidura leucodon*), samur, dovşan və s., quş növlərindən bildirçin (*Coturnix coturnix*), meşə göyərçini (*Columba oenas*), adi qur-qur (*Streptopelia turtur*), meşə cüllütü (*Gallinago gallinago*), göl qağayısı (*Chroicocephalus ridibundus*), saqqallı quzugötürən (*Gypaetus barbatus*), qara kərkəs (*Aegypius monachus*), leşyeyən ağkərkəs (*Neophron percnopterus*), iri yapalaq (*Bubo bubo*), bayquş (*Asio otus*), fısıldayan qu quşu (*Cygnus olor*), qara ağacdələn (*Dryocopus martius*), sarıköynək (*Oriolus oriolus*), meşə turağayı (*Lullula arborea*), çil qaratoyuq (*Turdus viscivorus*), Xəzər uları, kərkəs, kəklik və s. və s. yayılmışdır.

Milli Parkın ərazisində nadir növ olan Qafqaz maralı (*Cervus elaphus*) qorunur [Mustafayev, Mahmudov: 2016, s.95-110; http://eco.gov.az/az/77-milli-parklar; http://eco.gov.az/index.php?ln=az&pg=83].

Zaqatala DTQ - 1929-cu ildə Zaqatala və Balakən inzibati rayonlarının ərazisində, Böyük Qafqaz sıra dağlarının cənub mikroyamaclarında yaradılıb. Dəniz səviyyəsindən 650-3646 m hündürlükdə yerləşir. Əvvəllər ərazisi 23844 hektar olub. 2008-ci ildə isə hökumətin qərarı ilə sahəsi genişləndirilərək 47349 hektara çatdırılıb. Qoruğun yaradılmasında əsas məqsəd buradakı meşələrin əvəzedilməz torpaqqoruyucu və susaxlayıcı xüsusiyyətlərini saxlamaq, aşağı zonada yerləşən yaşayış məntəqələrini və əkin sahələrini seldən qorumaq, Böyük Qafqazın cənub yamacının təbii kompleksini, bitki və heyvanat aləmini mühafizə etmək olmuşdur. Qoruğun ərazisi meşələr, subalp və alp çəmənlikləri və yüksək sal qayalıqlardan ibarətdir. Aşağı və orta dağ qurşaqları (800-2200 m. d. s. h.) fısdıq, vələs və palıd meşələri ilə örtülüdür, yüksəklik artdıqca bunlar subalp, subalp çəmənlikləri (2400 m.), daha sonra isə alp çəmənlikləri (3200 m-ə kimi) və nəhayət, subnival və nival qurşaqları ilə əvəz olunurlar. Qoruğun ərazisi çox mürəkkəb və kəskin relyefə malikdir. Burada dağlar və yüksəkliklər dərin dərələr və daşlı-qayalı çay sahilləri ilə kəsilir.

Qoruğun şimal hissəsində yerləşən dağlar şiş zirvəli sal qayalardan ibarətdir. Zaqatala Dövlət Təbiət Qoruğunun ərazisi çox kəskin çay şəbəkəsi ilə kəsilir. Torpaq örtüyü əsasən dağ-çəmən, dağ-çəmən-meşə və qonur meşə torpaq tiplərindən ibarətdir. Qoruqda 1000-dən artıq bitki növünə təsadüf edilir. Meşə əmələ gətirən növlərdən fısdıq (*Fagus*), palıd (*Quercus*), vələs (*Carpinus*), cökə (*Tilia*), göyrüş (*Fraxinus*), şabalıd (*Aesculus*), qoz (*Juglans*), qaraçöhrə (*Taxus*), qarmaqvari şam (*Pinus hamata*) və s. göstərmək olar. Burada 32 növ məməli, o cümlədən 4500-dən çox Şərqi Qafqaz təkəsi, 1000 baş maral (*Cervidae*), 700 baş qarapaça (*Rupicapra rupicapra*) və 2000-ə yaxın çöl donuzu (*Sus scrofa*) vardır. Həmçinin qoruq ərazisində 89 növ köçəri və oturaq quşlar yaşayır [Əliyev, Həsənov, 1993: s.62-78; http://eco.gov.az/az/79-qoruqlar].

Türyançay DTQ - 1958-ci ildə 12344 hektar ərazidə yaradılmışdır. 2003-cü ildə hökumətin müvafiq qərarı ilə ərazisi iki dəfə genişləndirilib. Hazırda sahəsi 22488 hektardır. Qoruq Ağdaş, Oğuz, Yevlax və Qəbələ rayonlarının ərazisində yerləşir. Qoruğun yaradılmasında məqsəd Azərbaycanın arid meşə sahələrindən olan Bozdağın arid meşə lanşaft kompleksini qorumaqdır. Ərazisi qərbdən və şimal-qərbdən şərqə və cənub-şərqə doğru uzanan alçaq dağ tirələrdən ibarətdir. Ərazi kəskin parçalanmış relyefə malikdir. Relyefin paçalanması burada arid iqlim şəraitində bitki örtüyünün zəif inkişafı, torpaq örtüyünün isə az qalın və az münbitli olması ilə nəticələnmişdir. Quru subtropik iqlim Turyançay qoruğunun ərazisi üçün səciyyəvidir. Burada yay isti, qış isə mülayim-isti keçir. Ərazidə yarımsəhra və bozqır bitki formasiyaları yayılmışdır. Özünəməxsus arid bitki örtüyü vardır. Burada ağac və kol bitkilərindən ən çox ardıc (*Juniperus*) və saqqızağacı (*Pistacia lentiscus*), qismən də gürcü palıdı (*Quercus iberica*), gürcü ağcaqayını (*Acer ibericum*), qaratikan (*Paliurus*), şərq doqquzdonu (*Lonicera orientalis*), kiçik meyvəli gilas, nar (*Punica granatum*), murdarça (*Rhamnus*), sarağan (*Cotinus*), efedra (*Ephedra*) və s. inkişaf etmişdir. Qoruqda məməli heyvanlardan çöl donuzu (Sus scrofa), qonur ayı (*Ursus arctos*), porsuq (*Meles meles*), dələ (*Martes*), tülkü (*Vulpes*), dovşan (Lepus), çaqqal (*Canis aureus*), vaşaq (*Lynx*) və s. məskən

salmışlar. Sürünənlərdən müxtəlif növ ilanlara və kərtənkələlərə rast gəlinir. İlanlardan ən çox gürzəyə (*Vipera kaznakovi*), sularda isə Xəzər tısbağasına (*Mauremys caspica*) tez-tez rast gəlinir.

Burada quşlardan kəklik (*Perdix*), göyərçin (*Columba*), adi qur-qur (*Streptopelia turtur*), ağbaş Asiya kərkəsi (*Gyps fulvus*), leşyeyən qartal, qara kərkəs (*Aegypius monachus*), sərçə (*Passer*), qaratoyuq (*Turdidae*), zığ-zığ (*Garrulus glandarius*), payız bülbülü, dağ vələmirquşu, böyük və uzunquyruq arıquşu və s. kimi növlər məskunlaşmışlar. Adları Azərbaycanın «Qırmızı kitabı»na daxil edilmiş quşlardan şahinə (*Falco peregrinus*), berkuta (*Aquila chrysaetos*), çöl qartalına (*Aquila nipalensis*), həmçinin ayıya (*Ursus*) təsadüf edilir [Əliyev, Həsənov: 1993, s.117-132; http://eco.gov.az/az/79-qoruqlar].

İşıqlı Qaragöl DTQ - 1987-ci ildə yaradılmışdır. Azərbaycan Respublikasının Laçın rayonu ilə Ermənistan Respublikasının Gorus rayonu sərhədində, Qarabağ Vulkanik yaylasının cənub hissəsində böyük İşıqlı dağının (3552m) yamacında 2650-2700 m hündürlükdə yerləşir. Xüsusi maraq doğuran yüksək dağ göllərindən biridir. Alp landşaft kompleksinə malikdir. Ərazisi 240 hektardır. Ərazi üçün qışı quraq keçən soyuq iqlim tipi hakimdir. Burada illik yağıntının miqdarı 700 mm-dir. Qoruğun gölü əhatə edən quru ərazisi yüksək dağlıq qurşağın səciyyəvi alp çəmənliklərindən ibarətdir. Ərazidə çimli dağ-çəmən torpaqları yayılmışdır. Bu torpaqlar humusla zəngindir. Bitki nümunələrinin azlığı qoruğun quru sahəsinin kiçik olması, nadir və endemik bitkilərin onun sərhədlərindən kənarda qalması ilə əlaqədardır. Burada bitki örtüyünün inkişafına və zənginliyinə uzun illər qoyun sürülərinin otarılması və suvarmaya gətirilməsi nəticəsində tapdalanaraq korlanması xeyli mənfi təsir göstərmişdir. Bitki örtüyü əsasən alp çəmən bitkilərindən ibarətdir. Cənub hissədə üçyarpaq yoncanın (*Trifolium pratense*), şərq hissədə isə gəvənin (*Astragalus*) üstünlük təşkil etdiyi çəmənliklər inkişaf edir. Gölün suyu ərazinin hündürlüyü ilə əlaqədar bitkilərdən kasıb olub, burada yalnız amfibi qırxbuğumu (*Chulusium amphibium*) və qaymaqçiçəyi (*Ranunculus*) bitir. Qaragölün ekoloji şəraiti və suyunun spesifik xüsusiyyətləri burada canlı aləmin kasad olmasına səbəb olmuşdur. Bununla belə, gölün suyunda zooplanktonlara, dibində isə yanüzənlərə (Q*ammarus*) rast gəlinir [Əliyev, Həsənov: 1993, s.195-197; http://eco.gov.az/az/79-qoruqlar].

İlisu DTQ - 1987-ci ildə 9345 hektar ərazidə yaradılmışdır. Böyük Qafqazın cənub yamacında (Qax rayonu), Zaqatala və İsmayıllı qoruqlarının arasında, 700-2100 m hündürlükdə yerləşir. 2003-cü ildə Azərbaycan Respublikası Nazirlər Kabinetinin Qərarı ilə qoruğun ərazisi genişlən-dirilərək, 17381,6 hektara çatdırrılıb. Qoruğun təşkilində məqsəd təbii kompleksi daha yaxşı qorumaq və bərpa etmək, nadir və məhv olmaq təhlükəsi qarşısında qalan bitki və heyvanları qorumaq, onların sayını artırmaq, meşələrin əvvəlki vəziyyətini bərpa etmək, torpaqların eroziyaya uğramasının və sel hadisələrinin qarşısını almaqdır. Qoruğun adı Qax rayonundakı İlisu kəndinin adından götürülüb. Qoruğun ərazi Baş Qafqaz dağlarının dik yamaclı, çay dərələri vasitəsilə intensiv parçalanmış sahələri üçün səciyyəvi relyefə malikdir. Burada qışı quraq keçən soyuq iqlim və yağıntıları təxminən bərabər paylanan mülayim - isti iqlim tipləri hakimdir. İlisu qoruğunun ərazisində dağ-çəmən, dağ-çəmən-meşə, qonur dağ meşə, çürüntülü-karbonatlı dağ-meşə və qəhvəyi dağ-meşə torpaqları yayılmışdır.

İlisu qoruğunda dərman, endemik, nadir və nəsli kəsilmək təhlükəsi altında olan bitki növləri çoxdur. Ərazidə Şərq fıstığı (*Fagus orientalis*), Qafqaz vələsi (Carpinus caucasica), Şərq palıdı (*Quercus macranthera*), Litvinov tozağacı, Trautvetter ağcaqayını, quşarmudu, itburnu *(Rosa)*, böyürtkən *(Eubatus)*, əzgil *(Mespilus)*, dovşanalması (*Cotoneaster*), topulqa (*Crenata*), yabanı gilas (*Prunus avium*), zoğal (*Cornus)*, moruq (*Rubus*) və s. bitkilər yayılmışdır. Qoruq ərazisindəki bitkilərdən qaraçöhrə (*Taxus*) və Radde ağcaqayınının (*Acer raddei*) adları Qırmızı kitaba daxil edilmişdir.

Burada həmçinin 5 dəstəyə aid olan 35 növ məməli yaşayır. Bunlar dırnaqlılardan - nəcib maral (*Cervus elaphus*), cüyür (*Capreolus*), köpgər (*Rupicapra rupicapra*), təkə, çöl donuzu (*Sus scrofa*), yırtıcılardan - qonur ayı (*Ursus arctos*), canavar (*Canis*), tülkü (*Vulpes*), daş dələsi (*Martes foina*), meşə dələsi, gəlincik (*Mustela*), porsuq (*Meles meles*), yenot (*Procyon*), meşə pişiyi (*Felis silvestris silvestris*), vaşaq (*Lynx*), gəmiricilərdən – dovşan (*Lepus*), süleysinlər (*Gliridae*), siçanlar (*Muridae*), sincablar (*Sciurus*) və s., cücüyeyənlərdən – ağdiş, qonurdiş, kirpilər (*Erinaceinae*), nalburunlar (*Rhinolophidae*) və s. ibarətdir.

Qoruq ərazisində quşların 11 dəstəyə aid olan 90-a qədər növü yayılmışdır. Onların 60 növə qədəri sərçəkimilərə aiddir. Ərazidə həmçinin sürünənlərin 12 növü yaşayır. Onların 7 növ kərtənkələlərə, 3 növü təlxələrə, 1 növü ilanlara, 1 növü isə koramallara aiddir.

Qoruqdakı çaylarda balıqlardan çay forel (*Salmo trutta fario*) yaşayır. Digər balıqlardan Şərq qışovçusu, Kür şirbiti (*Barbus lacerta*) və Kür xramulyası (*Capoeta capoeta*) yalnız aşağı sərhəddindəki hissəsndə məskunlaşmışdır. Burada suda-quruda yaşayanlardan yalnız 5 növ qurbağa yayılmışdır

[Əliyev, Həsənov: 1993, s.78-84; http://eco.gov.az/az/79-qoruqlar].

Laçın DTY - 1961-ci ildə Laçın rayonunun ərazisində yaradılmışdır. Yasaqlığın yaradılmasında məqsəd buradakı məməliləri və quşları qoruyub artırmaqdır. Ərazisi 20000 hektardır.

Ərazi əsasən vələs (*Carpinus*), cökə (*Tilia*), ağcaqayın (*Acer*), palıd (*Quercus*) cinsli meşələrdən ibarətdir. Burada bir sıra heyvanların da məskunlaşması üçün əlverişli şərait vardır. Yasaqlıqda Bezoar keçisi (Capra aegagrus), Avropa cüyürü (*Capreolus capreolus*), çöl donuzu (*Sus scrofa*), boz dovşan (*Lepus europaeus*), boz canavar (*Canis lupus*), adi porsuq (*Meles meles*), meşə dələsi (*Martes martes*), qonur ayı (*Ursus arctos*) və s. məməlilərə, adi çil kəklik (*Perdix perdix*), Qafqaz tetrası (*Lyrurus mlokosiewiczi*) adi bildirçin (*Coturnix coturnix*), alabaxta (*Columba palumbus*) kimi quşlara rast gəlinir [http://eco.gov.az/az/80-yasaqliqlar].

Korçay DTY - 1961-ci ildə Xanlar və Goranboy rayonlarının ərazisində yaradılmışdır. Yaradılmasının əsas məqsədi Bozdağın təbii landşaftının, nadir və nəsli kəsilmək təhlükəsi qarşısında olan heyvan növlərinin qorunub saxlanılmasıdır. Yasaqlığın ümumi sahəsi 15.000 hektardır.

Ərazinin coğrafi relyefi düzənlik, təpəlik və alçaq dağlıq (Bozdağ) sahələrindən ibarətdir Ərazinin təbii landşaftı çoxillik kserofil ot və yovşanlı-efemerli və yovşanlı-şorangəli bitki formasiyalarından ibarətdir. Korçay vadisində seyrək yulğun kolları, qarğı və qamışdan ibarət bitki qruplaşmaları, Kür çayı boyu isə tuqay tipli meşələr yayılmışdır. Burada məməlilərdən ceyran (*Gazella subgutturosa*), boz dovşan (*Lepus europaeus*), adi tülkü (*Vulpes vulpes*), adi çaqqal (*Canis aureus*), boz canavar (*Canis lupus*), quşlardan adi çil kəklik (*Perdix perdix*), adi turac (*Francolinus francolinus*) və s. vardır [http://eco.gov.az/az/80-yasaqliqlar].

Qusar DTY - 1964-cü ildə Qusar rayonunda yaradılmışdır. Yasaqlığın yaradılmasında başlıca məqsəd buradakı bir sıra ov əhəmiyyətli məməli heyvanları və quşları qoruyub saxlamaqdır. Ərazisi 15000 hektardır. Meşə sahəsində fıstıq (*Fagus*), vələs (*Carpinus*), palıd (*Quercus*) cinslərinə aid olan ağaclar yayılmışdır. Kol bitkilərindən yemişan (*Crataegus*), zoğal (*Cornus*), armud (*Pyrus*), alça (*Prunus*), alma (*Malus*), itburnu (*Rosa*), böyürtkən (*Rubus*), qaratikana (*Paliurus spina-christi*) təsadüf edilir. Yasaqlıqda çöl donuzu (*Sus scrofa*), cüyür (*Capreolus capreolus*), canavar (*Canis lupus*), meşə pişiyi (*Felis silvestris*), qamış pişiyi (*Felis chaus*), çaqqal (*Canis aureus*), Amerika yenotu (*Procyon lotor*), boz dovşan (*Lepus europaeus*) kimi məməlilər, adi turac (*Francolinus francolinus*), adi çil kəklik (*Perdix perdix*), adi bildirçin (*Coturnix coturnix*), çöl göyərçini (*Columba livia*), yaşılbaş ördək (*Anas platyrhynchos*), fitçi ördək (*Anas crecca*) kimi quşlar yayılmışdır [http://eco.gov.az/az/80-yasaqliqlar].

İsmayıllı DTY - 1969-cu ildə yaradılmışdır. Yasaqlıq əsasən İsmayıllı, qismən də Qəbələ rayonlarının ərazisində yerləşir. Əvvəl ərazisi 34400 hektar olmuş, müvafiq dövlət qərarı əsasında 23437,85 hektara qədər azaldılmışdır. Yasaqlığın yaradılmasında əsas məqsəd burada məskunlaşmış heyvanların qorunması və saylarının artırılmasıdır. Ərazinin bitki örtüyü alp və subalp dağ-çəmən və meşə bitkilərindən ibarətdir. Meşə ilə örtülü sahələr əsasən vələs (*Carpinus*), fıstıq (*Fagus*) və palıd (*Quercus*) cinsli ağaclardan ibarətdir. Yasaqlığın ərazisində heyvanlar aləmi həm növ, həm də say etibarilə çox zəngindir. Burada quşlardan adi çil kəklik (*Perdix perdix*), adi bildirçin (*Coturnix coturnix*), adi turac (*Francolinus francolinus*), alabaxta (*Columba palumbus*), Qafqaz uları (*Tetraogallus caucasicus*), məməlilərdən boz canavar (*Canis lupus*), adi çaqqal (Canis aureus), vaşaq (*Lynx lynx*), meşə pişiyi (*Felis silvestris*), Amerika yenotu (*Procyon lotor*), meşə dələsi (*Martes martes*), boz dovşan (*Lepus europaeus*) məskunlaşmışdır [http://eco.gov.az/az/80-yasaqliqlar].

Zuvand DTY - 1969-cu ildə Lerik və Yardımlı rayonları ərazisində yaradılmışdır. Yasaqlığın yaradılmasında əsas məqsəd dağ-bozqır landşaftının bioloji müxtəlifliyinin qorunmasıdır. Sahəsi 15 000 hektardır. Zuvand zonasında dəniz səviyyəsindən 1400-2000 m yüksəklikdə, əsasən, dağ kserofit və bozqır, çəmən-bozqır bitkiləri yayılmışdır. Dövlət Təbiət Yasaqlığın ərazisində dovşanalması (*Cotoneaster sp.),* yemişan *(Crataegus sp.),* pallas murdarçası *(Rhamnus pal-lasi),* itburnu *(Rosa sp.)* kimi kol bitkilərinə rast gəlinir.

Ot bitkilərindən ərazidə qızılı gəvən *(Astragalus aureus),* acılıq *(Ephedra sp.),* yovşan *(Artemisia sp.),* kəklikotu *(Thymus sp.),* ağbığ *(Nardus sp,),* məryəmnoxudu *(Teucrium sp.), sığırquyruğu (Verbascum sp.),* boymadərən *(Achillea sp.),* şiyav *(Stipa sp.)* daha çox yayılmışdır.

Burada quşlardan əsasən adi çil kəklik (*Perdix perdix*), xınalı kəklik (*Alectoris chukar*), qartal (*Aquila* sp.) və qırğı növləri (*Accipiter* sp.); məməlilərdən Bezoar keçisi (*Capra aegagrus*), zolaqlı kaftar (*Hyaena hyaena*), çöl donuzu (*Sus scrofa*), boz dovşan (*Lepus europaeus*), boz canavar (*Canis lupus*), adi tülkü (*Vulpes vulpes*) və s. heyvanlar yayılmışdır [http://eco.gov.az/az/80-yasaqliqlar].

Qubadlı DTY - 1969-cu ildə Qubadlı və Laçın rayonlarının ərazisində yaradılmışdır. Qubadlı rayonunun şimal və Laçın rayonunun cənub hissəsində dağ-bozqır sahələrini əhatə edir. Bu yasaqlığın yaradılmasında məqsəd həmin ərazilərin heyvanlar aləmini, xüsusilə burada məskunlaşmış məməli heyvan növlərini (cüyür - *Capreolus*, çöl donuzu - *Sus scrofa*) və quşları (qırqovul - *Phasianus*) qorumaqdır. Sahəsi 20 000 hektardır. Ərazidə palıd (*Quercus*), vələs (*Carpinus*), ardıc (*Juniperus*), yemişan (*Crataegus*), itburnu (*Rosa*), böyürtkən (*Rubus*) cinslərinə aid ağac və kollardan ibarət seyrək meşəliklər və kolluqlar vardır.

Ərazinin ekoloji amilləri burada heyvanların normal məskunlaşmasına tam şərait yaratmışdır. Ərazidə çöl donuzu (*Sus scrofa*), qonur ayı (*Ursus arctos*), boz canavar (*Canis lupus*), adi çaqqal (*Canis aureus*), adi tülkü (Vulpes vulpes), Avropa cüyürü (*Capreolus capreolus*), boz dovşan (*Lepus europaeus*), adi porsuq (*Meles meles*), adi çil kəklik (*Perdix perdix*), adi qırqovul (*Phasianus colchicus*), adi turac (*Francolinus francolinus*), adi bildirçin (*Coturnix coturnix*) yayılmışdır [http://eco.gov.az/az/80-yasaqliqlar].

Ordubad DTY - 1969-cu ildə Naxçıvan Muxtar Respublikasının Ordubad rayonu ərazisində yaradılmışdır. Azərbaycan Respublikası Prezidentinin 2003-cü il 16 iyun tarixli Sərəncamı ilə Ordubad rayonunun inzibati ərazisinin 12131 hektar sahəsi Milli Park elan edilmişdir. Hal-hazırda yasaqlığın ərazisi 27869 hektardır. Yasaqlıq Zəngəzur dağlarının cənub-qərb yamacında yerləşir 1000-3900 m hündürlükləri əhatə edir

Yasaqlığın yaradılmasında məqsəd ərazidəki nadir və qiymətli heyvanları qoruyub artırmaqdır.

Ərazidə Bezoar keçisi (Capra aegagrus), Qafqaz köpgəri (Rupicapra rupicapra), daşlıq dələsi (*Martes foina*), boz canavar (*Canis lupus*), adi çaqqal (*Canis aureus*), adi tülkü (*Vulpes vulpes*) və s. məməli heyvan növlərinə, adi turac (*Francolinus francolinus*), adi qırqovul

(*Phasianus colchicus*), adi çil kəklik (*Perdix perdix*), adi bildirçin (*Coturnix coturnix*), alabaxta (*Columba palumbus*) və s. quş növlərinə rast gəlinir. Bitki örtüyü subnival qaya, alp, subalp və bozqırlaş-mış çəmənli, dağ kserofit bitkilərdən ibarətdir [http://eco.gov.az/az/80-yasaqliqlar].

Daşaltı DTY - 1981-ci ildə Şuşa şəhərinin ətrafında yaradılmışdır. Sahəsi 450 hektardır. Şuşa şəhəri və Şuşa rayonu Qarabağın, Azərbaycanın ən səfalı və füsunkar təbiətli, zəngin tarixi abidəli sahələrindən biridir. Onların qorunmasının, etalon ərazi kimi saxlanıl-asının təbiət və tarixi baxımdan mühüm əhəmiyyəti vardır. Yasaqlığın ərazisi zəngin meşə, kol və ot bitkilərinin təbii muzeyidir.

Burada palıd (*Quercus*), vələs (*Carpinus*), ağcaqayın (*Acer*), cökə (*Tilia*), göyrüş (*Fraxinus*), yemişan (*Crataegus*), itburnu (*Rosa*), zoğal (*Cornus*), əzgil (*Mespilus*), alma (*Malus*), armud (*Pyrus*), alça (*Prunus*) və s. ağac və kol bitkiləri inkişaf edir. Ərazi endemik və nadir bitkilərlə, həmçinin dərman əhəmiyyətli bitkilərlə zəngindir.

Burada Avropa cüyürü (*Capreolus capreolus*), çöl donuzu (Sus scrofa), daşlıq dələsi *(Martes foina*), boz dovşan (*Lepus europaeus*), boz canavar (*Canis lupus*), adi çaqqal (*Canis aureus*) və müxtəlif quş növləri məskunlaşmışdır [http://eco.gov.az/az/80-yasaqliqlar].

Qızılca DTY - 1984-cü ildə Gədəbəy Meşə Təsərrüfatının Qızılca meşəçiliyi sahəsində yaradılmışdır. Yasaqlığın yaradılmasında əsas məqsəd ərazinin təbiət kompleksini, xüsusilə məhv olmaq təhlükəsində olan heyvanlar və bitki nümunələrini qorumaqdır. Sahəsi 5135 hektardır.

Ərazidə meşə və dağ-bozqır bitkiləri inkişaf etmişdir. Burada meşəni əmələ gətirən əsas ağac cinsləri palıd (*Quercus*), fıstıq (*Fagus*) və vələsdir (*Carpinus*). Həmçinin ağcaqayın (*Acer*), göyrüş (*Oleeae*), dərman bitkiləri və endemik bitkilər yayılıb.

Yasaqlıqda cüyür (*Capreolus capreolus*), qonur ayı (*Ursus arctos*), çöl donuzu (*Sus scrofa*), dovşan (*Lepus*) kimi məməli heyvanlar və müxtəlif quşlar qorunur [http://eco.gov.az/az/80-yasaqliqlar].

Safari Parkı – Azərbaycan Respublikası Prezidentinin 6 dekabr 2016-cı il tarixli Sərəncamı ilə təsdiq edilən "Azərbaycanda ixtisaslaşmış turizm sənayesinin inkişafına dair Strateji Yol Xəritəsi"nə əsasən, Şamaxı rayonunun Pirqulu dağ-meşə sahəsində ümumi ərazisi 1200 hektar olan parkın 830 hektarlıq hissəsi beynəlxalq standartlara uyğun çəpərlənib və burada vəhşi heyvanlar üçün ekoloji qoruq - Şamaxı Safari Parkı yaradılıb. Parkın yaradılmasında məqsəd insanların təbiətə hörmətlə yanaşmasını stimullaşdırmaqdır. Qeyd etmək lazımdır ki, Latviya, Polşa, Slovakiya, Çexiya və Macarıstan təsərrüfatlarından nəcib maral (*Cervus elaphus*), xallı maral (*Cervus nipon*), Altay maralı, muflon (*Ovis musimon*), alkapa (*Vicugna pacos*), lama (*Lama glama*) və zubr (*Bison bonasus*) kimi genetik qiymətli heyvanlar - gətirilərək xüsusi mühafizə olunan əraziyə buraxılıb. Seleksiya və baytarlıq tədbirləri nəticəsində onların sayı 2-3 il ərzində iki dəfəyə yaxın artıb. Belə ki, istifadəyə verildiyi vaxt parkda 790 baş heyvan - nəcib maral, lan və muflon kimi genetik qiymətli heyvanlar var idi. Görülən seleksiya və baytarlıq tədbirləri nəticəsində bu heyvanların sayı hazırda 1000 başdan çoxdur [https://azvision.az/news/159456/samaxi-safari-parkina-ekskursiya-foto.html https://azertag.az/ru/xeber/Edinstvennyi_na_Kavkaze_safari_park_raspahnul_dveri_pered_posetitelyami___Reportazh_iz_SHamahi-1899558].

Qeyd etmək lazımdır ki, safari turizmi Afrikada yaranıb. "Safari" sözü Tanzaniyalıların dilindən götürülüb - səyahət etmək, yolçuluq, gəzinti mənasını verir. Bu cür turlar Afrikanın bir sıra ölkələrində Qərbi Avropadan və Amerikadan gələn imkanlı turistlərin maraqlarını yerli vəhşi fauna nümunələrinə cəlb etmək məqsədilə həyata keçirilib.

Ekoloji parkın daxili infrastrukturunun yaradılması istiqamətində də mühüm işlər görülüb. 36 kilometr uzunluğunda enli yol inşa edilib. Heyvanlar üçün çox sayda təbii daldalanacaqlar düzəldilib. Maral, lan və muflonları kənar təsirlərdən və yoluxucu xəstəliklərdən qorumaq

üçün beynəlxalq standartlara uyğun dəmir-beton dayaqlara hörülmüş 52 kilometr uzunluğunda çəpər çəkilib. Parkın ərazisində inzibati bina və qonaq evi tikilib.

Bu gün safari turizmi ovçuluq və ya fotoovçuluq, balıq tutmaq, açıq məkanda təbii halda heyvanları müşahidə etmək məqsədilə təşkil olunan turizm növü kimi məşhurdur. Ənənəvi safarilər heyvanları nəsli kəsilmək təhlükəsi ilə üz-üzə qoyduğu üçün son dövrlərdə təşkilatçılar ov məqsədli turlardan imtina edərək sırf fotosafarilərə istiqamətləniblər. Fotosafariləri "sakit ov" da adlandırırlar [https://president.az/az/articles/view-/29768]. Azərbaycanda fotosafari turlarının həyata keçirilməsi üçün geniş imkanlar vardır.

Tövsiyyə.

- Zaqatala, Göygöl, Qızılağac, və Hirkan DTQ-larınun Biosfer DTQ-na çevrilməsini faydalı hesab edirik.
- 30 il ərzində ermənilər tərəfindən işğal altında qalan Cəbrayıl və Kəlbəcər rayonlarının ərazilərində, əvvəllər bu bölgələrdə nəzərdə tutulmş xüsusi rejimli qoruq ərazilərinin təkilini vacib sayırıq [Azərbaycan Respublikasında ətraf mühitin və təbiəti mühafizə fəaliyyətinin vəziyyətinə dair Dövlət Məruzəsi, 1993: s.93; Əliyev, Həsənov, 1993: s.31].
- Vəhşi heyvanlar üçün "Safari Parkı" tipli ekoloji qoruqların digər bölgələrin dağlıq ərazilərində də yaradılması məqsədəuyğun olardı.

Sonuç.

Dağlıq və dağətəyi ərazilərdə yaradılmış qorunan ərazilərdə biomüxtəlifliyin saxlanması üçün geniş imkanlar vardır. Qoruq və yasaqlıqlarda, milli parklarda həm yerli, həm də əraziyə introduksiya edilmiş növlər dağ ekosistemində təbii birliklər şəklində yaşayırlar. Onların mühafizəsi müvafiq dövlət qurumları tərəfində təmin olunur və qoruna bilmələri üçün zəruri tədbirlər həyata keçirilir. Belə şəraitdə həm elmi tədqiqatların, həm də ekoloji maarifçilik işlərinin aparılması, ekoturizmin inkişaf etdirilməsidağ üçün geniş imkanlar vardır.

Ölkəmizin müxtəlif guşələrində xüsusi mühafizə olunan təbiət əraziləri - milli parklar, qoruqlar xarici turistlərin belə turlara cəlb edilməsində mühüm rol oynaya bilər. Ərazisinin böyüklüyünə, eləcə də heyvanlarının növ sayının çox olmasına görə nəinki Azərbaycanda, ümumiyyətlə, Qafqazda yeganə olan Şamaxı Safari Parkı da belə məkanlardandır.

Xəzər dənizinin yaxınlığı, təbiətin rəngarəngliyi Hirkan MP ərazisində ekoturizmin inkişafı və ətraf muhitin öyrənilməsi ucun əlverişli şərait yaradır.

Beləliklə, Azərbaycan Respublikasında ekoloji problemlərin həllinə yönəldilmiş strateji və tak-tiki tədbirlər biomüxtəlifliyin saxlanması və qorunması işlərinin beynəlxalq standartlara çatdırılması yönündə mühüm addımdır.

ƏDƏBİYYAT

Azərbaycan Xalq Cumhuriyyəti Ensiklopediyası. 2 cilddə, I c., Bakı: Lider, 2004, 440 s.

Azərbaycan Respublikasında ətraf mühitin və təbiəti mühafizə fəaliyyətinin vəziyyətinə dair Dövlət Məruzəsi / A.Ə.Mənsurovun redaktəsi ilə. Bakı: Ergün, 1993, 180 s.

Əliyev H.Ə., Həsənov X.H. Təbiətin keşiyində. Elmi-kütləvi əsər. Bakı: Maarif, 1993, 311 s.

İlham Əliyevin uğurlu ekoloji siyasəti / H.Bağırovun redaktəsilə. Bakı: Şərq-Qərb, 2008, 399 s.

Işğal olunmuş Azərbaycan ərazilərində ətraf mühitə və təbii sərvətlərə dağıdıcı təsiri müəyyənləşdirən (izləyən) operativ mərkəzin I hesabatı. Bakı: Ekologiya və Təbii Sərvəylər Nazirliyi, 2002, 100 s.

Mahmudov Y.M. Azərbaycan Respublikasında ətraf mühitin qorunması tarixi (1991-2011-ci illər). Monoqrafiya. Bakı: Füyuzat, 2022, 276 s.

Mustafayev Q.T., Mahmudov Y.M. Azərbaycan Respublikasinda ekoloji vəziyyətə kömək məqsədilə icra olunan tədbirlər. "İnsan və Biosfer" (MaB,UNESKO) Azərbaycan Milli Komitəsinin əsərləri. Bakı: Təhsil, 2016 №11, s.95-110

Prezident İlham Əliyev və birinci xanım Mehriban Əliyeva Şamaxı Safari Parkında olublar. (https://president.az/az/articles/view/29768)

Şamaxı Safari Parkına ekskursiya - FOTO (https://azvision.az/news/159456/samaxi-safari-parkina-ekskursiya-foto.html)

Тюркоглу Р. Единственный на Кавказе сафари-парк распахнул двери перед посетителями – Репортаж из Шамахи https://azertag.az/ru/xeber/Edinstvennyi_-na_Kavkaze_safari_park_raspahnul_dveri_pered_posetitelyami___Reportazh_iz_SHamahi-1899558

http://eco.gov.az/az/77-milli-parklar

http://eco.gov.az/az/79-qoruqlar

http://eco.gov.az/az/80-yasaqliqlar

BİR KÖPEKTE PREPİSYUM ENFEKSİYONUNDAN İZOLE EDİLEN FUNGAL VE BAKTERİYEL AJANLARIN TESPİTİ VE MOLEKÜLER KARAKTERİZASYONU

THE DETECTION AND MOLECULAR CHARACTERIZATION OF FUNGAL AND BACTERIAL AGENTS ISOLATED FROM PREPUTIUM INFECTION IN A DOG

Dr. Burcu KARAGULLE
Orcid no: 0000-0002-6628-4515
Firat University, Faculty of Veterinary Medicine, Department of Microbiology
Dr. Tutku Can ACISU
Orcid no: 0000-0002-0882-937X
Firat University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination

ÖZET

Bu çalışmada; bir köpeğin prepisyum enfeksiyonundan izole edilen patojen etkenlerin teşhisi, moleküler karakterizasyonu ve etkin bir tedavi protokolünün uygulanması amaçlandı. Samoyed ırkı 8 aylık bir erkek köpek, prepisyum çevresinde kirli bir görünüm ve penisten geldiği düşünülen kötü kokulu, koyu renkli, kıvamlı bir akıntı şikayetiyle Fırat Üniversitesi Veteriner Fakültesi Dölerme ve Suni Tohumlama Anabilim Dalı'na getirildi. Anamnezde akıntının yaklaşık olarak 1 ay önce başladığı, miktarının giderek arttığı ve genital bölgeden rahatsız edici bir kokunun yayıldığı öğrenildi. Hayvan sahibi, köpeğini damızlık olarak yetiştirdiğini ve dişisi ile çiftleştirmek istediğini ancak döl tutmadığını belirtti. Ürogenital bölge muayenesinde testislerin skrotum içerisindeki pozisyonlarının, büyüklüklerinin ve skrotal çapın normal görünümde olduğu belirlendi. Skrotal deri üzerinde herhangi bir lezyona rastlanmadı. Anamnez sonrası, prepisyumdan steril bir swap yardımıyla alınan örnek, aseptik şartlarda Mikrobiyoloji Anabilim Dalı'na gönderildi. Bakteri izolasyonu için %5 defibrine koyun Kanlı agar ve MacConkey agar yüzeyine ekim yapıldı ve 37 °C de aerobik ortamda 48 saat inkubasyona bırakıldı. Mantar izolasyonu için ise; Saboraud Dekstroz Agara ekim yapılarak 25 °C de 5-7 gün inkubasyona bırakıldı. Kanlı agar ve MacConkey agarda üreme olmazken, Saboraud Dekstroz Agarda *Penicillium spp*. izole edildi. Mantar tedavisine başlandı. (Oral yolla günlük 200 mg dozda olacak şekilde Flukonazol, topikal olarak Ketokonazol, ayrıca Hindistan cevizi yağı). Tedavi sonrası hayvanın şikayetlerinin hafiflediği ancak tamamen geçmediği belirtildi. Prepisyumdan tekrar örnek alınarak *Mycoplasma spp* ve *Staphylococcus epidermidis* izolasyonu gerçekleştirildi. İzolatlara DNA ekstraksiyonu yapılarak moleküler amaçlı 16s RNA cins-spesifik primerleri ile PCR analizi yapıldı. PCR ürünleri sekans analizi ile konfirme edildi. Bunun üzerine *Mycoplasma spp*. ve *S. epidermidis* için antibiyotik tedavisine başlandı. Kontrole geldiğinde akıntının kesildiği, prepisyumdaki kirli görüntünün ortadan kalktığı ve hayvanın çiftleşerek sağlıklı yavru sahibi olduğu görüldü. Bu, ülkemizde üreme hastalığı olan bir köpekte *Penicillium spp, Mycoplasma spp* ve *S epidermidis*'in birlikte izole edilerek tedavi edildiği ilk olgu sunumudur.

Anahtar Kelimeler: *Mycoplasma spp, S. epidermidis*, Köpek, PCR, Prepisyum

ABSTRACT

In this study; it was aimed to diagnose, molecular characterization and application of an effective treatment protocol of pathogenic agents isolated from a dog's preputium infection. An 8-month-old Samoyed male dog was came to Fırat University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination with a dirty appearance around the preputium and a foul-smelling, dark-colored, viscous discharge thought to come

from the penis. In the anamnesis, it was learned that the discharge started approximately 1 month ago, its amount gradually increased and an unpleasant odor spread from the genital area. The owner said he wanted to mate his dog but it was not fertilized. In the urogenital region examination, it was determined that the position of the testicles in the scrotum, their size and scrotal diameter were normal. No lesions were found on the scrotal skin. After anamnesis, the swab taken from the preputium was sent to the Microbiology Department for bacteriological analysis under aseptic conditions. Swap sample was directly inoculated on blood agar plates containing 5.0% sterile defibrinated sheep blood and incubated at 37.5 °C for 24-48 hours under aerobic conditions. Fungi were isolated on Sabouraud dextrose agar incubated at 25 °C for 5-7 days. While there was no growth on Blood agar and MacConkey agar, *Penicillium spp* was isolated on Saboraud Dextrose Agar. Fungus treatment was started. (Orally 200 mg daily dose of Fluconazole, topically Ketoconazole, also coconut oil). After the treatment, the animal's complaints did not completely disappear. *Mycoplasma spp* and *Staphylococcus epidermidis* isolation were performed by taking samples from the preputium again. DNA extraction was performed on the isolates and PCR analysis was performed with molecular 16sRNA genus-specific primers. PCR products were confirmed by sequence analysis. Following this, antibiotic treatment was started for *Mycoplasma spp* and *S. epidermidis*. When it came to the control, it was seen that the flow stopped, the dirty appearance in the preputium disappeared and the animal mated and had healthy offspring. This is the first case report in our country in which *Penicillium spp*, *Mycoplasma spp* and *S. epidermidis* were isolated and treated together in a dog with reproductive disease.

Keywords: *Mycoplasma spp, S.epidermidis*, Dog, PCR, Preputium

GLP AGONIST – AN INNOVATIVE APPROACH TO TREAT TYPE 2 DIABETES MELLITUS

Dr. Subia Jamil[1]
Amber Nawab[2]
Salma Jabbar[3]
Simran Haresh Kumar[3]
Wajeeha Samar[3]
[1] Department of Pharmacology, Jinnah University for women. Karachi Pakistan
[2] Department of Pharmaceutics, Jinnah University for women. Karachi Pakistan
[3] Department of Pharmacy, Jinnah University for women. Karachi Pakistan

ABSTRACT

Diabetes mellitus (DM) is a chronic metabolic disease characterized by inappropriate increased levels of glucose in blood. It has become a serious cause of public health issue worldwide. The prevalence of this disease has increased at an astounding rate. For individuals having type 2 diabetes mellitus a number of new therapeutic approaches have been introduced. For instance; anti-diabetic agents, pancreatic implants, gene therapies and so on so far. The modern world is looking forward for novel therapies to treat type 2 diabetes. One of the latest discoveries regarding anti-diabetic drugs has been discussed briefly in this review study which is Glucagon like peptide 1 receptor agonist (GLP-1 RA) that has been proven significant in patients having T2DM. GLP-1 RA act on incretin hormone which facilitates glucose regulation by stimulating insulin secretion in a glucose dependent manner while suppressing glucagon secretion. By comparing it with other antidiabetic agents correspondingly it has been observed that it does not show weight gain, complete occupancy of beta-cell function and hyperglycemia as the most common anti diabetic agents produce. Other than there convincing significance these agents are more expensive comparative to other anti diabetic medications, like sulfonylureas (SFUs), metformin, and TZDs. But its Once-weekly formulations may also improve patient adherence and patient compliance. GLP-1 RA show lesser side effects like nausea, vomiting, and diarrhea. The reason behind this study is to highlight the metabolic, glycemic, possible weight modification effects and safety of GLP-1 receptor activator (GLP-1 RA) on glycemic management as well as on numerous metabolic parameters for this purpose we went through different available authentic researches and collected data.

KEYWORDS: Comparison, Diabetes mellitus, GLP AGONIST, Hyperglycemia, Therapeutic approach

HERBAL MEDICINES: CONSUMER PERCEPTIONS AS A TREATMENT APPROACH DURING PANDEMIC

Amber Nawab[1]
Javeria Sheikh[2]
Subia Jamil[3]
Sehar Iqtidar[4]
Zubia Begum[5]

[1]Assistant Professor, Department of Pharmaceutics, Faculty of Pharmacy, Jinnah University for Women, Karachi
[2] Lecturer, Department of Pharmaceutics, Faculty of Pharmacy, Jinnah University for Women, Karachi
[3] Associate Professor, Department of Pharmacology, Faculty of Pharmacy, Jinnah University for Women, Karachi
[4] Corporate Lecturer, Department of Pharmaceutics, Faculty of Pharmacy, Jinnah University for Women, Karachi
[5]Lecturer, Department of Pharmacology, Faculty of Pharmacy, Jinnah University for Women, Karachi

ABSTRACT

INTRODUCTION

Herbal medicine popularly known as phytomedicine, originally prepared using different plant components with reported activities. Generally, a societal myth is where people believe that products labeled "natural" are always safe and good for them; while is not necessarily true. The main objective of this research article is to evaluate general population's attitude and perception regarding herbal medicines and to discover the limit of utilization to underline the major factors affecting the usage of herbal medicines.

METHODOLOGY:

The opted methodology is cross-sectional survey based study, conducted on general population of residents of Karachi, the designed questionnaire limits the age (18-36), locality and the education criteria. According to Z-Test statistical analysis the sample size was randomly selected at 95% confidence intervals. The designed questionnaire is divided in three appendices for the evaluation of attitude of individuals towards herbal medicines, their general perception and their preference of herbal medicines over any other treatment approaches. Using different statistical tools, the results were identified.

RESULT:

The evaluated results suggest that 68.6% prefer allopathic treatment over herbal treatment as they give fast recovery, while 76.7% individual consider if herbal treatment re concomitantly given with allopathic treatment they have observed better results with better safety profile and have experienced lesser adverse effects.

CONCLUSION:

The results showed that general population depending upon different criteria have very mixed attitude and perception regarding use of herbal medicines, and consider that herbal treatment are comparatively safer than allopathic treatment but prefer allopathic treatment over herbal treatment because faster recovery.

Keywords: Herbal medicines, phytomedicines, conventional treatment.

DETERMINATION OF RIBOFLAVIN PRODUCTION OF *LACTOCOCCUS LACTIS* MH3 AND *PEDIOCOCCUS ACIDILACTICI* MH10

Sama SULTAN[1]
Harun ÖNLÜ[2]
Özlem OSMANAĞAOĞLU[1]
1 Ankara Universty, Departmen of Biology, Ankara, Türkiye
2 Muş Alparslan University, Department of Molecular Biology and Genetics, Muş, Türkiye

ABSTRACT

Introduction: Riboflavin is a water-soluble vitamin produced by all plants and most microorganisms and has a significant impact on human health. Vitamin B2 is a basic micronutrient that performs numerous physiological functions in the human body and its deficiency leads to a variety of health disorders. Lactic acid bacteria (LAB) are an important group of microorganisms used in the food industry that provide health benefits. They are also considered safe as they have been used for many years as starter cultures in the production of fermented foods. Some of the LAB strains are known to produce riboflavin. In this study, two different isolates from a group of lactic acid bacteria *Lactococcus lactis* MH3 and *Pediococcus acidilactici* MH10 strains riboflavin production levels were investigated.

Material Method: The presence of the *ribC* gene in the *Lactococcus lactis* MH3 strain and the presence of the *ribB* and *ribG* genes, which are required for riboflavin synthesis, in all isolates were first examined by the PCR method using specific primers. Supernatants of strains grown for 24 hours at 37°C in riboflavin assay medium (Difco, France) were used for riboflavin isolation. After verifying the presence of the gene regions, the riboflavin production levels of the strains were determined by HPLC by using Zorbax XDB C18 column (250 mm x 4.6 mm i.d., 5µm).

Results and Discussion: It was found that *Pediococcus acidilactici* MH10 had a riboflavin production capacity of 0.11 ng/ul and strain *Lactococcus lactis* MH3 of 0.22ng/ul. Thus, the strains used in the study were found to have vitamin B2 production capacity. It was determined that the strains showed weak riboflavin production ability. This production amount can be improved by using both metabolic and genetic engineering methods.

Keywords: Riboflavin, Lactic acid bacteria, *Pediococcus acidilactici, Lactococcus lactis*

UNIVERSITY INTERDISCIPLINARY COLLABORATIVE METHODS IN THE FIELD OF DERMATOLOGY

Assist Prof. Dr. Felıcıa ANDREI[1]
Assist. Prof. dr. Toma Ana Olivia[2]
Assist. Prof. dr. Avram Ștefania[2]
Assist. Prof. dr. Popa Valentin Tudor[2]
Lecturer Dr. Gencia Ioana[2]
Assoc.Prof.Dr. Cristodor Patricia Liana[2]
1 University of Medicine and Pharmacy "Victor Babes", Faculty of Pharmacy, Department I Timisoara, Romania
2 University of Medicine and Pharmacy "Victor Babes", Faculty of Medicine, Deparment of Dermatology

ABSTRACT

The field of dermatology is part of the Medical Sciences and is integrated into the University part of training and research of the medical and pharmaceutical students of different levels and specialities.

For the students of the faculty of medicine there is within our University a special department dedicated to dermatology integrated within the university clinic which serves a wide population from the city of Timișoara but also from the western part of Romania. Also the students of the faculty of dental medicine have the study of dermatology during one semester in their curricula. The third faculty within the Timișoara University of Medicine and Pharmacy, respectively the Pharmacy faculty has a special discipline dedicated to Dermatopharmacy and Cosmetology.

The aim of our study was to research elaborate and implement effective methods of study and collaboration between the three different faculties of our University. As method of study we requested the collaboration of students from the Batchelor and Master study levels, as well as from those enrolled in various forms of postgraduate studies (residency, postgraduate course, doctorate, postdoctoral research). As a study method we used online questionnaires but also physical ones when needed.

The results showed the need to apply a collaborative interdisciplinary integrated study so that all participants obtain maximum results in their study or research activity. additionally, the participation of the IT department was requested in order to create an online interface or even a mobile application, so that all students can use both for entering data and for obtaining valuable information in this field.

Keywords: dermatology, dermatopharmacy, cosmetology

MALARIAL PARASITE INFECTION AND GENOTYPE RELATIONSHIP AMONG PATIENT ATTENDING FEDERAL MEDICAL CENTRE KATSINA

Habiba Zakari Haruna
Department of Biology, Umaru Musa Yar'adua University Katsina, Nigeria

ABSTRACT

There are increased evidences about the relationship between malaria prevalence and genotype, even though the range of such relation is yet to be understood. Malaria parasite infection and genotype of patients attending Federal Medical Centre (FMC) Katsina were investigated. Using blood samples Infection status and genotype were screened using standard techniques. Their demographic characteristics were determined using questionnaires. Chi-square test was used to determine the degree of relationship between malarial parasite infection and genotype. Out of 400 consented patients examined, 193 patients (48.3%) were affected by malaria at varying degree of parasitemia.

According to the gender, the prevalence of malaria was found to be (48.3%) among female respondents compared to their male counterparts with 48.1% ($P>0.05$). The results further showed that the subjects with normal genotypes are significantly more infected compared to those with S gene. The occurrence of malarial infection among the participants with normal (AA) genotype is comparatively higher (53.8%) compared to the carrier (AS) participants with 41.7% and those with sickle cell disease (SS) having the least with 33.3%. Conclusively people with AS and SS genotype have more genetic advantage toward resistance to Malarial parasite infection. However, more scientific reasons require further elucidation.

Keywords- Malarial Parasite, parasitemia, Genotype, Blood, Patients, Genes, Genetics

ISOLATION OF SKIN INFECTIOUS BACTERIA, ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS AGAINST *Staphylococcus aureus, Staphylococcus epidermidis* and *E.coli* STRAINS

Syeda Kahkashan Kazmi*
Naheed Kauser
Ayisha Aman
Aqsa Idrees
Rashida Rahmat Zohra
Anoosha Sohail
Saima Komal

Department of Biotechnology, Jinnah University for Women, 5C Nazimabad, Karachi –P.O Box 74600

ABSTRACT

In the present time both males and females are equally sensitive about their skin. Wrinkles, sun burn, itching, black heads, white heads and uneven tone of the skin are the collective issues related to skin. Extensive use of antibiotics for skin diseases is restricted due to the development of worse antibiotic resistance. Natural components such as fruits, herbs, minerals, leaves, rose water and flowers are now becoming part of skin care and treatment products. Skin care and treatment products are now becoming more advanced, with herbal ingredients to kill infectious microbes and prevent from skin diseases. Plants contain active ingredients crucial to uphold skin health. Skin products with herbal ingredients are nowadays evolving as a suitable solution to the existing skin problems. In this study anti-bacterial activity of eight different plant extracts was performed against three isolated skin infectious bacteria i.e. *Staphylococcus aureus, Staphylococcus epidermidis* and *E.coli* strains. Among eight plant extracts, *Azadirachta indica, Curcuma longa, Santalum album, Fumaria indica, Swertia chirayita and Citrus reticulata blanco* showed good inhibition of bacterial growth. Afterwards face wash formulation from these extracts was prepared against skin infections.

BENZYL ACETATE DERIVATIVES: SYNTHESIS AND ANTIBACTERIAL ACTIVITY

Bilal Umar
Umaru Musa Yar'adua University, Faculty of Natural and Applied Sciences, Department of Chemistry, Katsina, Nigeria
Yusuf Hassan
Umaru Musa Yar'adua University, Faculty of Natural and Applied Sciences, Department of Chemistry, Katsina, Nigeria
Abdulhamid Ahmed
Umaru Musa Yar'adua University, Faculty of Natural and Applied Sciences, Department of Biology, Katsina, Nigeria

ABSTRACT
Antibiotics continue to be less effective in treating common infectious diseases as noted in the difficulty encountered in the treatment of infections like tuberculosis, pneumonia, gonorrhea etc. The cause to this challenge is attributed to the new resistance mechanisms that are emerging and spreading globally. Hence, the need to discover and develop new generations of antibiotics is an urgent endeavor. Compounds containing ester functional group(s) are found to be involved in many important metabolic processes and are present in the structure of some vital drugs effectively used to manage some medical conditions. In this work, five benzyl acetate derivatives were synthesised, characterised and tested against *Staphylococcus aureus* and *Shigella spp*. The antibacterial activity determined using disc diffusion method was recorded as the zones of inhibition of the bacterial growth in comparison with a standard drug (amoxicillin disc, 0.5 g/L) as a positive control. It was found that all the five compounds exhibited some activities against the two organisms at 100µg/ml. The highest zones of inhibition of *Staphylococcus aureus* and *Shigella spp* were found to be 16.5 mm and 17.5 mm respectively. However, a comparison of the activity of the compounds with the control drug which inhibited the growth of *Staphylococcus aureus* and *Shigella spp* with zones of 42 mm and 36 mm, respectively, revealed that the compounds were far less active than the control drug.

Key words: Benzyl acetates, antibacterial activity, *Staphylococcus aureus, Shigella spp*

ANTI-OXIDANT AND ANTI-BACTERIAL PROPERTIES OF *Beta vulgaris* L (BEET ROOT) ROOT EXTRACT

Dogun Ojochogu[1]
Emmamoge Orewere[2]
Ogenyi Ruth Ajibola[1]
Longchi Satkat Zacheaus[3]
[1]Department of Science Laboratory Technology, Federal College of Forestry, Jos, Plateau State
[2]Department of Horticulture and Landscape Technology, Federal College of Forestry, Jos, Plateau State
[3]Department of Plant Science and Biotechnology, University of Jos, Nigeria

ABSTRACT
The aim of this study was to evaluate the antioxidant and antibacterial potential of *Beta vulgaris* L. root extract against some pathogenic bacteria. The plant was purchased and processed. Cold maceration was used for extraction and standard scientific methods were used for phytochemical analysis. The hydrogen peroxide scavenging activity was used to test for the antioxidant activity and the disc diffusion technique was used for antibacterial susceptibility testing. The phytochemical results showed that the plant possessed metabolites such as steroids, terpenoid, flavonoids and phenols, but saponins, tannins and glycosides were absent in all the extracts. The Ethanolic extract of *Beta vulgaris* had the highest percentage yield at 8.17% while the aqueous extract had a lower percentage yield at 1.65%. Antioxidant activity results showed that there was a significant difference in antioxidant activity at $P \leq 0.005$. The aqueous extract showed the highest antioxidant activity in the range of 10.76±0.05 - 98.10±0.32 %, followed by the ethanolic extracts at 10.33±0.05 - 85.73±0.04 %. The antibacterial screening revealed that the bacterial strains were resistant to both root extracts at the various concentrations. The result of this study supports the use of this plant to reduce oxidative stress when consumed.
Key Words: Antioxidant, Antibacterial, *Beta vulgaris*, Phytochemicals.

INTRODUCTION
Beta vulgaris (Beetroot) is a crop belonging to the Chenopodieceae family having, bright crimson colour. It is famous for its juice value and medicinal properties, and known by several common names like beet chard, spinach beet, sea beet, garden beet, white beet and chukandar in Hindi (Yashwant, 2015).*Beta vulgaris* are small herbaceous plants with broad dark green leaves. *Beta vulgaris* is one of the richest sources of folate. It is also a source of fiber, potassium, manganese, iron, vitamin C and a number of other vitamins and minerals. (Rauha *et al.,* 2005).
In different parts of the world especially in Africa and Asia with high incidence of diseases, people have learnt to manage the problem using plants which are God's gift of nature. Various advances in scientific research on the use of plant and herbs have brought the beneficial aspect of traditional medicine and the rational for their uses to the limelight (Okpuzor *et al.,* 2008). Medicinal plants have always been used by humans to treat pathogenic diseases. About 80% of the population in developing countries currently uses medicinal plants to treat pathogenic diseases (Aziz *et al*.,2018) Medicinal plants contain phytochemicals that are biologically important and therapeutic (Khan *et al*., 2011). These medicinal plants contain

natural antioxidants that are effective in preventing oxidative stress and as a defense mechanism against pathogens and herbivores; plants synthesize various groups of compounds that can be used as substitutes for synthetic drugs (Khan *et al*., 2011). Phytochemicals found in various organs of medicinal plants can be used as natural antioxidants, substitutes for synthetic antibiotics, food preservatives, and dietary supplements.

METHODOLOGY

Sample Collection and Identification

Beta vulgaris root was bought from farin gada market in Jos metropolis, the plant was identified and Voucher specimens were prepared and stored at the herbarium unit at the Federal College of Forestry Jos, Plateau state. The root was air dried at room temperature (25^0c) for two weeks and pulverized into fine powder using a mortar and pestle the powder was sieved and stored in air tight bottles until when needed

Extraction of beet root

Extraction was performed using cold maceration by taking 100 g and macerating with 500 ml of ethanol and distilled water for 72 hours. The extract was filtered through filter paper (Whatman No.1) and dried in a water bath (Parekh and Chanda, 2007).

Phytochemical Determination

Test for Alkaloids

About 0.5g of each extract was stirred with 3ml of 1% aqueous hydrochloric acid on a steam bath; 1ml each of the filtrate was treated with a few drops of Mayers reagent, Dragendorff's reagent and Picric solution.Precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloid in the extract. (Sofowora, 2008)

Test for Saponins

About 0.5g of each plant extract was shaken with water in a test tube. Frothing which persist on warming was taken as preliminary evidence for the presence of saponins.

Test for Tannins

About 0.5g of plant extract was stirred with 1ml of distilled water and filtered; ferric chloride was added to the filtrate. A blue-black, green, or blue green precipitate indicated the presence of tannins.

Test for Anthraquinones

Borntrager's test was used for the detection of anthraquinones, 0.5g of each extracts was taken into a dry test tube and 5ml of chloroform was added and shook for 5 minutes. The extract was filtered, and the filtrate shaken with an equal volume of 100% ammonia solution. A pink violet or red colour in the ammonical layer (lower layer) indicated the presence of free anthraquinones.

Test for Cardiac Glycoside

100 mg of the extract was dissolved in 70% alcohol and filtered. About 3 drops of lead sub-acetate was introduced into the filtrate and filtered. The filtrate was extracted with 10 mls of choloform in a separating funnel and concentrated to dryness. The resulting residue was dissolved in 1ml of glacial acetic acid containing one drop of Ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring obtained at the interphase indicates the presence of a deoxysugar characteristic of cardenolides.

Test for Steroid and Terpenes

A little quantity of each extract was dissolved in chloroform, and 1ml of acetic anhydride was added, then two drops of concentrated Sulphuric acid was added. A pink colour which changes to bluish green on standing is indicative of the presence of steroid and terpenes.

Test for Flavonoids

5 ml dilute ammonia was added to 5 ml of the extract and then 5 ml concentrated sulfuric acid was added. Formation of a yellow colour shows the presence of flavonoids.

Test for Carbohydrates

100 mg of each extract was dissolved in 3ml of distilled water and mixed with a few drops of Molisch reagent (10% solution of napthol in alcohol) then 1 ml of concentrated sulphuric acid was carefully added down the side of the inclined tube so that the acid formed a layer beneath the solution. A white colour at the base indicated the presence of carbohydrates.

Antioxidant Activity Assay

The in-vitro antioxidant activity was carried using the hydrogen peroxide scavenging activity. 600 µml of hydrogen peroxide was dispensed in test tubes, 100 µml of the different concentration of the beet root extracts were added and the volume was made up to 4 ml using phosphate buffer. An identical reaction mixture was set up without samples serving as control. Test tubes were incubated for 10 minutes at room temperature. Absorbance was measure at 230 nm and the total percentage scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of control-Absorbance of samples}}{\text{Absorbance of control}} \times 100$$

Source of Micro-Organism

The bacterial strains which are *Salmonella typhii, Staphylococcus aureus* and *Escherichia coli* were obtained from the Veterinary Research Institute Vom. The organisms were collected in suspension of nutrient broth (NB).

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Disk diffusion technique

Antimicrobial susceptibility testing was performed on clinical isolates using the disc diffusion technique as described by Nair and Chanda (2005). Bacterial inoculum was prepared from subcultures as follows. One-day-old bacterial colonies were suspended in culture medium and turbidity adjusted to 0.5 McFarland standard. Solidified nutrient agar plates were inoculated with bacteria using a sterile swab technique. Discs impregnated with 250 mg/ml, 125 mg/ml, 62.5 mg/ml, and 31.25 mg/ml extract were placed on the inoculated plate and incubated at 37°C for 24 hours, locating the zone of inhibition at the nearest position measured up to Millimeters (mm).

Data Analysis

The experiment was carried out in triplicates and the data obtained was subjected to analysis of variance (ANOVA). Significance was taking at $p \leq 0.05$ using graph pad prism version 8.

RESULTS

Table 1: Phytochemical Screening of different oil of *A.indica* Fractions

Constituents	Aqueous	Ethanolic
Tannins	-	-
Saponins	_	-
Steroids	_	-
Terpernoids	+	+
Flavonoids	+	+
Alkaniod	+	-
Phenols	+++	++
Protein/amino acids	+	-
Acid	+	+
Anthroquinone	+	+
Coumaris	+	+

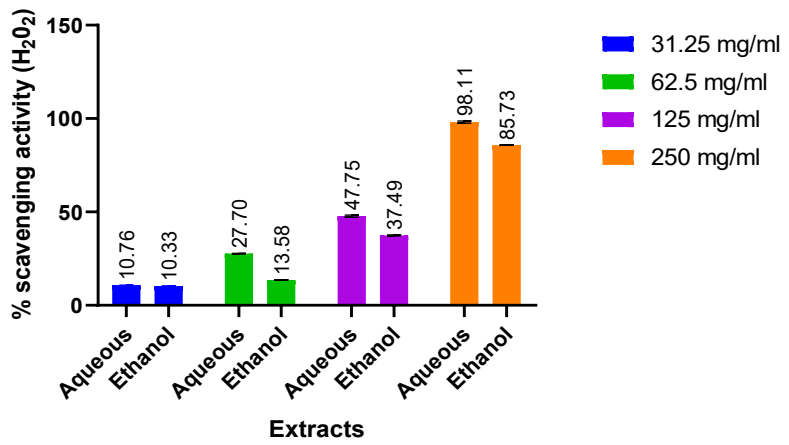


Figure 1: The antioxidant activity of the different extracts of beet roots.

Table 2: Antibacterial activity of the aqueous root extract of Beta vulgaris against *E.coli, S.typhi and S.aureus*

Organism	250mgml	125mgml	62.5mgml	31.25mgml	Control
E.coli	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	24±3.51
S.typhi	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	27±0.79
S.aureus	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	15±1.73

Table 3: Antibacterial activity of the Ethanolic root extract of Beta vulgaris against *E.coli, S.typhi and S.aureus*

Organism	250mgml	125mgml	62.5mgml	31.25mgml	Control
E.coli	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	24±3.51
S.typhi	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	27±0.79
S.aureus	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	15±1.73

DISCUSSION

Plants extracts contain different phytochemicals with various biological activities that can be of value in both medical and veterinary practice, the evolution of drug resistance in most microorganism have necessitated the need to find new and cheap starting materials that possess anti-microbial, antioxidant and anti-inflammatory potential, therefore this research was carried out to evaluate the anti-oxidant and antibacterial activities of *Beta vulgaris* (beet root) on some clinical isolates. Extraction results showed the highest recovery for the ethanol extract at 8.17% and the lowest recovery for the aqueous extract at 1.65%. This result is due to differences in the polarity of the solvents in which most of the secondary metabolites are soluble in. The results of the phytochemical showed that the plant possesses metabolites such as steroids, terpenoid, flavonoids and phenols, but saponins, tannins and glycosides were absent in all the extracts. This finding is consistent with other researchers who reported the presence of these metabolites in ethanol, acetone, and beet extracts (Jasna *et al*., 2001; kaur 2001; Riboli and Norat 2003).

The result of the antioxidant activities showed that at $P \leq 0.005$ there was a significant difference in the antioxidant activity. The aqueous extracts had the highest antioxidant activity of between 10.76±0.05 - 98.10±0.32 followed by the ethanolic extracts 10.33±0.05 - 85.73±0.04. This could be due to the high phenolic content of the plant. The result of the antibacterial activity studies revealed that both the aqueous and ethanolic extracts of beet root did not have any antibacterial activity on the test organism unlike like the control drug

gentamycin that had a broad spectrum of activities on the test organism with zones of inhibition of 24±3.5lmm, 27±0.79mm and 15±1.73mm for *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* respectively.

The result of this findings is in disagreement with the work of others (Kujala *et al*.,2001; Will 2000; Azeredo 2009; Jasna *et al*., 2011). Who have reported that the different extracts of *Beet vulgaris* have antibacterial activities on *Pseudomonas aerogenosa, Escherichia coli Bacillus subtilis* and *Staphylococcus aureus*. The result of this research could be related to genetic and environmental factors affecting the secondary metabolism of this species, harvesting time of the biological material in different developmental stages, interaction with *microorganism* and insects and different postharvest techniques are suggested by (Morais and Castanha 2012 and Camiloffi *et al.,* 2015). The resistant plasmid in the test organism could be responsible for the inability of the extract to inhibit the growth of the organism.

CONCLUSION

The results of this research showed that beet roots contains a lot of metabolites which could be used as sources of new antimicrobial agents particularly in reducing free radicals in the body. Therefore the consumption of this root is useful in reducing oxidative stress.

REFERENCES

Azeredo, H.M. (2009). Betalains: properties, sources, applications, and stability – a review. International Journal Food Science and Technology, 44: 2365–2376.

Aziz, M.A., Adnan, M., Khan, A.H., Shahat, A.A., Al-Said, M.S., and Ullah, R. (2018).Traditional uses of medicinal plants practiced by the indigenous communities at Mohmand Agency, FATA, Pakistan. J Ethnobiol Ethnomed. (*14*) 1–16. pmid: 29316951

Camiloffi, T., Ferarrese, L., Bortollucci, W.G., and Goncalues, J.E. (2015). Essential oil of fruits and vegetables to lnuitro control of insect. *Journal of medicinal plants*: 1021-1030

Chanda, S. and Baravalia, Y. (2010). Novel leads from herbal drugs for infectious skin diseases. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. 1: 451-456.

Jasna, M., Čanadanović-Brunet, Sladjana, S. S., Gordana S. Ć., Jelena, J. V., Sonja, M. D., Siniša, L. M., and Dragoljub D. C. (2011). Antioxidant and Antimicrobial Activities of Beet Root Pomace Extracts. *Czech Journal of Food Science* Vol. 29: 575–585.

Kaur, C. and Kapoor, H.C. (2001). Antioxidants in Fruits and Vegetables - The Millennium's Health. *International Journal of Food Science and Technology* Vol. 36: 703-725.

Khan, A.M., Qureshi, R.A., Ullah, F., Gilani, S.A., Nosheen, A., Sahreen, S.(2011).Phytochemical analysis of selected medicinal plants of Margalla hills and surroundings. *J Med Plant Res.* 5: 6017–6023.

Kujala, T., Loponen, J., and Phlaja K. (2001). Betalains and phenolics in red beetroot (Beta vulgaris) peel extracts: extraction and characterisation. Zeitschrift für Naturforschung – C, 56: 343–348.

Morais, L.A.S., Castanha, R.F. (2012). Chemical composition of sweet basil essential oil naturally submitted to Planococcus citri infestation. *Journal of Horticulture 30*(2):52178-52182.

Nair, R., Kakiriya, T.,and Chanda, S. (2005). Antibacterial activity of some selected Indian medicinal flora. *Turk J. Bio* 29: 41 – 47.

National Committee for clinical Laboratory Standards (NCCLs 2008).performance standard student for Antimicrobial susceptibility testing. Ninth information supplement NCCLs document M 100-59 National Committee for clinical laboratory standard wayle, P.A.

NCCLs (1993) performance standard for antimicrobial disc susceptibility test. Approved standard. NCCLs documents M2-A5, National Committee for Chemical Laboratory Standard Pennsylvania, USA.

Okpuzor, J., Adebesin, O., Ogbunugafor, H., and Amadi, I. (2008). The potential of medicinal plants in sickle cell disease control: A review. Int. J Biomed. Health Sci., *4*(2), 47-55.

Rauha, J.P., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T., Pihlaja, K., Vuorela H, and Vuorela, P. (2005). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int. J. Food Microbiol. 56:3-12.

Riboli, E., and Norat, T. (2003): Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. American Journal of Clinical Nutrition, 78: 559–569.

Rinaldoni, A.N., Palatnik, D.R., Zaritzky, N., and Campderrós, M.E. (2014). Soft cheese-like product development enriched with soy protein concentrates. *LWT-Food Sci Technol.* 2014; 55:139–147. doi: 10.1016/j.lwt.2013.09.003.

Sahreen, S., Khan, M.R., Khan, R.A, and Hadda, T.B. (2015).Evaluation of phytochemical content, antimicrobial, cytotoxic and antitumor activities of extract from Rumex hastatus D. Don roots. {BMC} Complement Altern Med. 15. pmid: 26138389

Sofowora, E.A. (2008). *Medicinal Plants and Traditional Medicines in Africa.* University of Ife press, Nigeria. p. 1-23.

Will, F., Bauckhage, K., and Dietrich, H. (2000). Apple pomace liquefaction with pectinases and cellulases: Analytical data of the corresponding juices. European Food Research and Technology, 211: 291–297.

Yashwant, K. (2015). Beetroot: A Super Food International Journal of Engineering Studies and Technical Approach. *1*(3), 20 - 26

TOXICITY STUDIES AND *IN VIVO* ANTIPLASMODIAL ACTIVITIES OF LEAF AND STEM BARK EXTRACTS OF *LANNEA MICROCARPA*

[*1]R. K Mustapha
[2]O. A. Adoum
S. Y. Mudi[2]
[3]M. Aliyu
[3]U.S. Abdussalam
[1]Department of Chemistry, Yusuf Maitama Sule University, Kano
[2]Department of Pure and Applied Chemistry, Bayero University, Kano
[3]Department of pharmacology and therapeutics, Bayero University Kano

ABSTRACT
Malaria is one of the most serious infectious diseases found in the tropics and subtropics. *P. falcifarum* resistance to almost all anti malarial drugs has necessitated the search for anti malarial compounds. The antiplasmodial effects of crude leaf and stem bark extracts of *L. microcarpa*, a plant utilized by traditional healers in Northern Nigeria to treat malaria and other ailments, were investigated in this work. The LC_{50} was used to assess cytotoxicity (lethality concentration). The median lethal dosage (LD_{50}) was calculated by administering different doses of the extract (100–4000 mg/kg) intraperitoneally to three groups of three mice each for 24 hours and observing the animals for physical symptoms of toxicity. Three models were employed to assess the extracts' antiplasmodial effectiveness *in vivo* against the rodent malaria parasite *Plasmodium berghei*: suppressive, curative, and repository. The extracts were found to show significant toxicity to brine shrimp larvae of *Artemia salina* in the brine shrimp lethality test with LC_{50} values of 289.02 and 303.4, respectively. Excitation, paw licking, and death were symptoms of acute toxicity of the extracts based on physical and behavioral observations. Death was recorded at 1000mg/Kg of the stem bark extract and the LD_{50} is ≤100mg/Kg body weight while for the leaf extract it was estimated to be ≤150mg/Kg with no death record. *In vivo* evaluation revealed that the extracts significantly (P 0.05) reduced parasite count in all models, with high average percentage inhibition of parasitaemia (66.01 %, 69.27 %, 52.41 %, 70.14 %, 57.85 %, and 61.17 %) seen in both *L. microcarpa* leaf and stem bark extracts, which was comparable to that produced by chloroquine and artesunate, respectively (64.31 % & 93.10 %). Both extracts had moderate antiplasmodial activity and were non-toxic to rats and brine shrimps. This finding validates the plant's traditional use in malaria treatment.

Key words: Malaria, Cytotoxicity, Acute toxicity, *L. microcarpa*, brine shrimps.

POSSIBLE SOURCES OF CLUSTERED REGULARLY INTERSPACED SHORT PALINDROMIC REPEATS IN *XANTHOMONAS* SPECIES

Svjetlana Tesic[1]
Elena Kyrova[2]
Aleksandr Ignatov[1]*

[1]People's Friendship University of Russia, Moscow, Russia
[2]Russian Institute of Plant Protection, St. Petersburg, Russia

ABSTRACT

Bacteria of the genus *Xanthomonas* are the causal agents of many plant diseases with significant threats to agricultural production. Understanding the adaptation of *Xanthomonas* species to new host plants and monitoring world-wide expansion are of interest for integrated disease management and seed production at the global level. Some repeated genetic elements in the Xathomonas genome can be used to assess the genetic diversity and evolution of bacteria.Clustered regularly interspaced short palindromic repeats (CRISPR) have been used before for comparison of *X. citri* and generating the genealogy of the spoligotypes, i.e., the absence/presence patterns of CRISPR spacers (Bellanger et al., 2022). Using consensus CRISPR repeat sequences (Bellanger et al., 2022), we identified the presence of major CRISPRs in 40 postulated *Xathomonas* species using available NCBI GenBank genomes. Moreover, we analyzed the presence of these CRISPRs in other bacteria and found the most related long CRISPRs array with CRISPR-associated proteins Cas1 and Cas2 in *Steroidobacter denitrificans* strain DSM 18526 (CP011971.1). *S. denitrificans* is a species of Gammaproteobacteria, Nevskiales, family Steroidobacteraceae. Significant genetic distance (30%) between the CRISPR fragment of *S. denitrificans* (approximately 3000 bp) and homologs in Xanthomonas species suggests that the CRISPR array in this species could be an ancestor of CRISPRs in some xanthomonads. This work presents a framework for further analyses of the CRISPR loci's roles in the speciation of the genus *Xanthomonas* and the global spread of the pathogen.

Keywords: CRISPR; epidemiology; evolution; *Xanthomonas*

Ref.: *Bellanger N, Dereeper A, Koebnik R. Clustered Regularly Interspaced Short Palindromic Repeats in Xanthomonas citri—Witnesses to a Global Expansion of a Bacterial Pathogen over Time. Microorganisms. 2022 Sep;10(9):1715.*

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL PROPERTIES OF *NYMPHAEA LOTUS* L. LEAVES EXTRACTS

Maryam Saddiq
Umaru Musa Yar'adua University, Faculty of Natural and Applied Sciences, Department of Chemistry, Katsina, Nigeria
Ahmed Salisu
Umaru Musa Yar'adua University, Faculty of Natural and Applied Sciences, Department of Chemistry, Katsina, Nigeria
Kamaluddeen Kabir
Umaru Musa Yar'adua University, Faculty of Natural and Applied Sciences, Department of Microbiology, Katsina, Nigeria
Yusuf Hassan
Umaru Musa Yar'adua University, Faculty of Natural and Applied Sciences, Department of Chemistry, Katsina, Nigeria

ABSTRACT
Plants have potential therapeutic value and this give them the ability to cure many ailments and infectious diseases. The present study investigated the phytoconstituents and antimicrobial properties of *Nymphaea lotus* leaves extracts against some clinical isolates. The extract was prepared by maceration using ethanol (90 % v/v). The crude extract was further fractionated into various fractions using different solvents viz; *n*-hexane, chloroform, ethyl acetate and ethanol. Among all the solvents, the ethanolic fraction showed the highest extraction yield and this clearly revealed that the extraction efficiency favored the highly polar solvent of ethanol. The qualitative phytochemical test carried out revealed the presence of alkaloids, terpenoids, steroids, glycosides, tannins, saponins and flavonoids which are believed to be responsible for the antimicrobial activities observed in the various fractions. The antimicrobial activity test was evaluated by agar disk diffusion method against the following clinical isolates; *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. The results revealed that *E.coli* and *P.aeruginosa* were susceptible to all the fractions with the highest zone of 18 and 16 mm respectively in the ethanol soluble fraction. *S. aureus* was moderately susceptible to the fractions with the zone of 14 mm while little activity was observed in the ethyl acetate soluble fraction against *C. albicans.* The study revealed that the ethanolic fraction has the highest activity and as such, it can be used to treat diseases caused by these organisms. Hence, *N. lotus* leaves can be a promising source of naturally occurring antibiotics; therefore, the study justifies some of the claims made by the traditional healers.
Keywords: *Nymphaea lotus*, Maceration, Phytoconstituents, Antimicrobial, Isolates.

INSECTICIDAL ACTIVITY OF ENDOPHYTIC FUNGAL EXTRACTS FROM *Lantana camara* LEAVES AGAINST MALARIA VECTOR *Anopheles gambiae*

Nafisatu A. Muhammad[1*]
Abdullahi J. Muhammad[2]
Fatima Idris Sambo[3]
[1]Department of Chemistry, Yusuf Maitama Sule University, Kano. Nigeria
[2]Department of Biology, Kano University of Science and Technology, Wudil, Nigeria
[3]Department of Biology, Yusuf Maitama Sule University, Kano. Nigeria

ABSTRACT
Mosquitoes are the vectors of several life-threatening diseases in humans such as malaria, yellow fever, chikungunya fever. The present study explored the effect of two endophytic fungal extracts isolated from the leaves of *lantana camara* on larvicidal, pupicidal and adulticidal activity against *Anopheles gambiae*. *Fusarium oxysporum* and *fusarium solani* were isolated from the leaves of *lantana camara* and identified based on the morphological characteristics of the colonies and microscopically using lacto phenol cotton blue staining technique. The ethyl acetate extract was obtained by liquid-liquid extraction of the fermented fungus in potato dextrose broth medium. Extract of *Fusarium oxysporum* recorded the highest activity with LC_{50} values of 177.38, 234.84 and 582.94 ppm for larvicidal, pupicidal and adulticidal respectively while the extract of *fusarium solani* proved to be less active with LC_{50} values of 411.77, 475.72 and 937.99 ppm of above-mentioned activities respectively. These results suggest that ethyl acetate extract of *Fusarium oxysporum* have the potential to be used as an eco-friendly approach for vector control programme.
Keywords: Insecticidal, Endophytic fungi, Activity, *Lantana camara*, Leaves.

CHELATE ASSISTED PHYTOREMEDIATION OF LEAD AND COPPER FROM SOIL IRRIGATED WITH MUNICIPAL WASTE WATER USING JATROPHA CURCAS

Dayo, L. Yusuf
Department of Pure and Industrial Chemistry Umaru Musa Yar'adua University, Katsina, Nigeria

ABSTRACT
Numerous hazardous compounds have been introduced into the soil via water used for irrigation of crops. These toxic compounds could be organic or inorganic in nature. Of most concern are toxic heavy metals which can affect man, plant and soil microorganisms. Phytoremediation is an eco friendly solar driven technology used for the decontamination of heavy metals from the soil. In this study, the ability of J. curcas in the extraction of Lead and Copper from soil irrigated with municipal waste water was investigated. Due to the limited bioavailability of some metals like Pb, Ethylene diamine-tetra-acetic acid (EDTA) was applied to the soil to facilitate the solubilisation and transport of the metals to the plant roots. The seeds of J. curcas were planted in pots containing 1kg of soil obtained from vegetable garden and irrigated with municipal waste water. EDTA (5ml of 1mmol) was added on the 10^{th}, 11^{th} and 12^{th} week. In the control, EDTA was not added. The plants were harvested after 13 weeks of planting. The average number of leaves and shoot heights were recorded. The Pb and Cu contents in the roots and shoots of the plants in both soils were also analysed. The results obtained showed that the application of EDTA increased the average number of leaves and shoot heights of J. curcas when compared to control. The results obtained also showed that EDTA influenced the uptake of metals by the tissues of the plant when compared to control. Phytoextraction ability was assessed in terms of bio concentration factor (BCF) and transfer factor (TF). Based on the TF and BCF values, J. curcas can be used as a good candidate for the stabilization of Pb and Cu in the soil.
Key words: Phytoremediation, Municipal waste water, EDTA.

PHYTOREMEDIATION OF LEAD AND CHROMIUM CONTAMINATED SOIL USING JATROPHA CURCAS

Dayo, L. Yusuf
Department of Pure and Industrial Chemistry, Umaru Musa Yar'adua University, Katsina, Katsina State, Nigeria
Samaila, M. Batagarawa
Department of Pure and Industrial Chemistry, Umaru Musa Yar'adua University, Katsina, Katsina State, Nigeria
Ibrahim Sada
Department of Pure and Industrial Chemistry Umaru Musa Yar'adua University, Katsina, Katsina State, Nigeria

ABSTRACT
The implications of illicit disposal of improperly treated wastes containing toxic heavy metals into the soil has been a great concern as this serve as a threat to human, animals and the environment. These toxic heavy metals find their way into plants and can affect the growth of plants by inhibiting seed germination and further development of seedlings. This in turn affects the overall productivity of plants. This has necessitated the cleanup of soils for effective farming using a sustainable method. Phytoremediation is a plant based solar energy driven technology that uses plants and its associated microbes for the restoration of polluted soil. In this study, the effects of different concentrations (10, 20 and 30ppm) of Lead (Pb) and Chromium (Cr) on seed germination and seedling growth of Jatropha curcas was investigated in pot experiment as well as the potential of using this plant for the remediation of these metals. Seeds of J. curcas were sown in different pots each filled with 1kg of soil and watered with tap water. Each pot was spiked separately with Pb and Cr salts at different concentrations (10, 20 and 30ppm). J. curcas seeds were also sown in pots filled with 1kg uncontaminated soil as control. The plants growth were monitored and harvested after 13 weeks. Pb and Cr accumulation in the roots and shoots of the plants were analysed. The results obtained showed that the average number of leaves and shoot height of J. curcas decreased with an increase in metal concentration in the soil. The results also showed that the uptake of Pb and Cr by the roots and shoots of J. curcas was concentration dependent. Uptake of metals by the plants decreased as the metal levels in the soils increased. Highest accumulation of Pb and Cr was observed in the roots and shoots of the plants sown in 10mg/kg of Pb and Cr contaminated soils respectively compared to other concentrations tested. The results showed that J. curcas sown in pots amended with 10mg/kg Pb, 10 and 20mg/kg Cr metal concentrations accumulated more metal in the roots than the shoots while in other tested concentrations, J. curcas accumulated more metals in the shoot. The BCF was less than 1 in all tested concentrations for both metals. The TF values obtained showed that at 10mg/kg Pb concentrations, Pb was stabilized in the soil and Cr was also stabilized in the soil at 10 and 20mg/kg Cr dose while at 20 and 30mg/kg Pb dose, Pb was translocated to the aerial parts of the plant and 30mg/kg Cr dose, Cr was also translocated to the aerial part of the plant.
Key words: Heavy metals, Phytoremediation, Translocation factor, Bio concentration factor.

I. Introduction
Soil contamination by various inorganic and organic compounds as a result of urbanization and industrialization has been a worldwide concern and this is a threat to food safety, human health the ecosystems, surface and ground waters (Li et al., 2009). Heavy metals are among

the most serious environmental pollutants due to their high toxicity, abundance and ease of accumulation by various plant and animal organisms, (Dadgne., 2017) unfortunately, little technology is known and applied for the cleanup of these pollutants, hence, their persistence in the environment (Azeez et al., 2020). The term ‘‘heavy metal’’ refers to any metal and metalloid element that has a relatively high density ranging from 3.5 to 7 g cm^3 with atomic number greater than twenty. These metals become noxious to living organism and the environment when they exceed their permissible limits (Ambika et al., 2016, Ahalaya et al, 2003, Ravindra et al., 2014). Heavy metals include cadmium (Cd), chromium (Cr), zinc (Zn), nickel (Ni), copper (Cu) and lead (Pb) (Morais et al., 2012; Monisha et al., 2014). The two different ways that heavy metals enter the environment are from natural and anthropogenic sources. Heavy metals get into the environment naturally from the weathering of mines, seepage from rocks into water, windblown dusts, volcanic activity, forest fires etc (Ahmadpour et al., 2012, Wei et al., 2008) which are less harmful (Muthusaravanan et al., 2018; Rashim el al., 2013) compared to anthropogenic sources such as smelting, mining, military activities, emissions from power stations, fuel production, incineration of wastes and waste disposal, application of fertilizers, pesticides and herbicides (Brooks et al., 1992; Jadia et al., 2009; Helmisaari et al., 1995; Zarcinas et al., 2004). All these have increased the levels of metals beyond background values which is a threat to human health and agricultural soil since these metals are non biodegradable thereby causing toxicity (Muthusaravanan et al., 2018; Jadia et al., 2009; Muhammad et al., 2008).

Some metals such as Co, Cu, Fe, Mn, Mo, Ni and Zn are essential for normal growth and metabolism but could be toxic when in excess (Ambika et al., 2016). Metals such as As, Cd, Hg, Pb or Se are non essential to plant as they do not perform any known physiological function in plants (Ambika et al., 2016, Rascio et al., 2011). These non essential metals and are taken up by plants alongside essential metals and since metals are non biodegradable, they accumulate in plants tissues and adversely affect plants when they exceed acceptable limits (Ambika et al., 2016). Heavy metals pollution has adverse effects on various parameters that affect plant quality and yield. They alter the size and activity of the microbial community as well as soil microbial properties (Singh et al., 2011, Yao et al., 2003). This leads to a decrease in the decomposition of organic matter in the soil, hence, less soil fertility. Enzyme activities needed for plant metabolism are also affected in the soil due to reduced microorganisms’ activities. All these effects consequently affect plant growth and yield or in some cases, plant death. (Ambika et al., 2016; Singh et al., 2011). Numerous remediation techniques such as excavation landfill of the top contaminated soils etc. have been developed for the reclamation of contaminated soils or to reduce the metal concentration in soils in the last two decades but are not sustainable or are not practically feasible especially on large scale contaminated farmland due to their extremely high cost, labour intensive generation of secondary waste and lack of a long-term solution (Danh et al., 2009; Prasad., 2003). Scientists are now studying a new approach, called Phytoremediation, a plant-based technology that enhances environmental clean-up by using green plants and their associated microbes to remove environmental pollutants or to render them harmless (Chigbo., 2013). The concept of Phytoremediation involves the ability of some plant species to accumulate higher quantities of toxic metals in their roots and translocated to the aerial parts of the plants without causing toxicity to their metabolic processes. It is efficient and cost-effective with high public acceptance and environmentally friendly aspects (Ahmadpour et al., 2012). This research work tries to investigate the potential of Jatropha curcas in the removal of lead (Pb) and chromium (Cr) from soils spiked with different concentrations of Pb and Cr.

II. Materials and methods

A. Soil sampling and treatment

The soil used for spiking of heavy metals and the control soil sample was collected within the premises of Umaru Musa Yar'adua University. Surface soil (0 – 15 cm) samples were collected at ten different locations using decontaminated soil probe. The soil samples were thoroughly mixed to obtain a representative sample; air dried for four days then crushed into fine powder and made to pass through 2mm mesh sieve.

B. Soil spiking

The soil sample was spiked with different concentrations (0, 10, 20 and 30mg/kg) of Pb and Cr separately in different containers. This was achieved by measuring 1, 2 and 3ml of $Pb(NO_3)_2$ solution from 1000mg/kg stock solution into different 250ml conical flasks and diluted with 100ml of deionised water. Each 100ml of different concentration of Pb solution was used to spike 1kg of soil sample to achieve 10mg/kg, 20mg/kg and 30mg/kg of Pb spiked soils respectively. The different concentrations of Cr spiked soils were prepared from $K_2Cr_2O_7$ using the same method. The spiked soils were kept in polyethylene bags and incubated in a dark room for 4 weeks to mimic contaminated soil and to also allow it to equilibrate and promote adsorption of the added metals. The soil samples were then air dried and sieved through a 2mm mesh sieve prior to the experiments. The parent soils were characterized in terms of physicochemical properties (pH, textural analysis, soil organic matter and cation exchange capacity).

C. Experimental design

The experiment was divided into two parts. The first part studied the effects of heavy metals on seed germination and further seedling growth of J. curcas sown in heavy metals spiked soils. This was done by monitoring the average number of leaves and shoot height of the plant compared to control. The second part studied the Phytoremediation potentials of Jatropha carcus in the removal of Pb and Cr from heavy metal spiked soils. The experiment consisted of 3 treatments (10, 20 and 30ppm) for each metal and control. All experiments were in triplicates and placed in the Biological Garden of Umaru Musa Yar'adua University, Katsina.

D. Phytoremediation Studies

The Phytoremediation experiment was carried out by filling different perforated plastic pots of 25cm diameter and 30cm height with 1.0 kg of heavy metal spiked soil sample. Each pot consists of different doses (10mg/kg, 20mg/kg and 30mg/kg) of Pb and Cr spiked soils. Seeds of Jatropha curcas were planted in each plastic pot containing the spiked soil. The control experiment contained soil without heavy metal treatment and all were watered with tap water every other day. The number of leaves and shoot heights of the plants were monitored. The plants were harvested after thirteen weeks of planting and the concentration of Pb and Cr in the roots and shoots of the plants were determined after thirteen weeks of planting.

E. Treatment of plant samples

The plants were harvested after 13 weeks of planting and were brought to the laboratory. These plants were washed thoroughly with tap water and later with deionised water to remove earthy impurities and any other form of dirt. This was to ensure that only the metals absorbed by the plant will be analysed. The washed plants were separated into roots and shoots, dried in open air for 2 days then in the oven at a temperature of 80°c for 5hrs. The dried plant samples were grinded to a fine powder using a cleaned ceramic pestle and mortal and sieved using a 2mm mesh sieve. The fine powdered sample of each part of the plant were stored and labelled in an acid cleaned container and kept for further analysis.

F. Digestion of Plant Samples

This was done by weighing 0.5g of the powdered samples of each part of the plant (roots and shoot) separately in into a $100cm^3$ digestion flask followed by the addition of 10 cm^3 of aqua

regia (a mixture of 3 parts concentrated HCl to 1 part concentrated HNO_3). This was heated on a hot plate in a fume cupboard, until a clear solution was obtained. The digest was diluted with de-ionised water, filtered into a 100cm^3 volumetric flask using Whatman No. 4 filter paper and then made up to mark with more de-ionised water (Kisku et al, 2000).

G. Elemental analysis

Microwave plasma Atomic emission spectrophotometer (MPAES) was used to determine the concentrations of Pb and Cr present in the roots and shoots of the plants used for the experiments.

H. Bio-concentration factor (BCF) Index

The ratio of heavy metal concentration in whole plant tissues to that in the soil was determined using the formula: $BCF = \frac{\text{metal concentration in root (mg kg} -1)}{\text{metal concentration in soil (mg kg} -1)}$ (Yoon et al., 2006; Cluis., (2004)

I. Transfer Factor (TF)

The capability of plants to take up heavy metals in their roots and to translocate them to their above-ground parts (shoots). This was calculated using the formula:

$TF = \frac{\text{metal concentration in shoot (mg kg} -1)}{\text{metal concentration in root (mg kg} -1)}$ Marchiol et al,. (2004)

J. Statistical analysis

The analysis was done using paired sample t test. This was done to compare the means of heavy metal in the plant tissues sown in spiked soil samples and control. The statistical soft ware used was statistical package for social sciences (SPSS).

III. Results and discussion

A. Physicochemical Parameters of Soils

The physical and chemical properties of soils give information about the availability and toxicity of metal contaminants in the soil therefore, its determination is important. The textural analysis of the soil showed a high percentage of sand (83%) and lower percentage of clay and silt (10 and 7%) therefore classifying the soil as loamy sandy. Soils with high sand contents and low levels of silt and clay are known to have high permeability and poor retention capacity for both water and metals (Wuana et al., 2010) which is noticeable in this soil sample. Also, the soil organic matter content (1.32%) and CEC (4.80) were quite low due to the high percentage of sand in the soil. Soil organic matter is an important indicator for judging soil fertility (Sen et al., 2019) and CEC is a very important soil property that influences the soil's ability to hold onto essential nutrients and to allow for easy exchange of cations between its surface and solution (Wuana et al., 2010). The pH value for the soil was 6.2 which is slightly acidic according to The US Department of Agriculture Natural Resources Conservation Services (1998) classification and this are within the pH range of agricultural soils.

Table 1.0: Physicochemical Parameters of Soil.

Physicochemical parameters	Values	Method of analysis
Particle size (%) Clay Sand Silt	 10.00 83.00 7.00	Hydrometer method
Soil texture	Loamy Sandy	
Soil pH	6.2	Potentiometric method

Moisture content (%)	49.47	Gravimetric method
Soil organic matter (%)	1.32	Walkley - Black
Cation Exchange Capacity, CEC ($cmol/kg^{-1}$)	4.80	Ammonium Acetate Method

B. The growth and toxicity symptoms of J. curcas in Pb and Cr spiked soils

Visual assessment of the response of J. curcas to soils spiked with different doses (10, 20 and 30mg/kg) of Pb and Cr showed that an increase in heavy metal concentration from 10 to 30mg/kg had negative effects on some of the seeds and seedling growth of the plant. Some of the effects of high doses of heavy metals observed on J. curcas are delayed seed germination, death of some seeds and seedlings. Loss of leaves and reduced growth were later observed in the seedlings and these effects increased as the concentration of the metals increased from 10 to 30mg/kg. This was due to the toxic effect of these heavy metals on the seedlings at high concentration resulting in changes in physiological and biochemical processes in plants. These observations are accordance with the report of Évio et al., (2008) who observed toxicity symptoms such as chlorosis, necrosis and reduced biomass in plants grown in all the soils contaminated with heavy metal.

The average number of leaves and shoot height of J curcas planted in different pots filled with soils spiked with different concentrations of Pb (10, 20 and 30mg/kg) and harvested after a period of twelve weeks are shown in Table 2.0. The average number of leaves of J. curcas in Pb spiked soils decreased as the level of Pb in the soil increase and also decreased with respect to control. The average number of leaves decreased in the order 9, 5.50, 4.75 and 3.50 in control, 10, 20 and 30ppm Pb spiked soils respectively. The average shoot height of J. curcas sown in 10mg/kg Pb spiked soil increased significantly ($P < 0.05$) over the control height (26.00cm) while that of 20 and 30mg/kg decreased. Within the three tested Pb concentrations, it was observed that J. curcas attained the highest shoot height of 27.50 in 10mg/kg Pb. Further increase in Pb level in the soil to 20 and 30ppm resulted in a significant decrease ($P < 0.05$) in the average shoot height of the plant of J. curcas to 21.75 and 12.50cm respectively. Lina et al., (2018) reported that Pb toxicity on plants inhibits root growth because it inhibits cell division that occurs at the root tip. The reduced shoot height observed in J. curcas with an increase in Pb concentration is similar to that obtained by Cokkizgin et al., (2010) where an increase in Pb concentration from 0.5 to 4.5mM caused significant decrease in germination percentage, plumule length and radicle length of lentil as the concentration increased.

Increase in Cr concentration from 10 to 30mg/kg inhibited the shoot development of J. curcas when compared to control. The plant attained a height of 26.00cm in control which reduced to 23.00, 19.00 and 13.33cm in 10, 20 and 30mg/kg Cr spiked soils respectively. The highest number of leaves was observed in the control (9) and the least (4) in 30ppm Cr spiked soil. Cr is a toxic heavy metal that interferes with several metabolic processes causing toxicity to the plants such as reduced root growth (Datta et al., 2011). In this study, increase in Cr concentration depressed plant growth. This result is in accordance with the result of Datta et al., (2011), where an increase in Cr concentration significantly affected the root and shoot length of the four wheat cultivars (Triticum aestivum L) tested. Ehsan et al., (2016) observed stunned growth and height decreased in Vinca (Vinca rosea L.) with higher levels of Cr treatments i.e. (50, 60 and 70 mg/kg). The reduced root growth could be due to inhibition of root cell division and root elongation due to accumulation of chromium in the vacuoles of the root cells.

TABLE 2.0: GROWTH PERFORMANCE of J. curcas in Pb and Cr spiked soils

	Pb		Cr	
Treatments	Average Number of Leaves	Plant height (cm)	Average Number of Leaves	Plant height (cm)
10mg/kg	5.50	27.50	7	23.00
20mg/kg	4.75	21.75	6	19.00
30mg/kg	3.50	12.50	4	13.33
Control	9	26	9	26.00

C. Concentration (mg/kg) of metals in the root and shoot of Jatropha curcas

Lead concentrations in roots and shoots of J. curcas harvested from different pots filled with 1kg of soil each spiked 10, 20 and 30 mg/kg of Pb salt are presented in Figure 1, Table 3. The results obtained from this experiment showed that the level of Pb in the root of J. curcas sown in 10mg/kg Pb spiked soil was higher than the root value of the control. The root of J. curcas in 10ppm Pb spiked soil accumulated 2.56mg/kg Pb while the control accumulated 0.53mg/kg. This gives about 383% increase when compared to control. Further increase in Pb dose to 20 and 30mg/kg reduced the Pb levels in the roots to 0.04 and 0.09mg/kg respectively when compared to control. This gives about 92% and 83% decrease with respect to root control value. The shoot Pb level of the plant in all tested concentration decreased with respect to control. The shoot accumulated 0.24, 0.07 and 0.09 in 10, 20 and 30ppm Pb spiked soils compared to shoot control value with 0.45mg/kg. This amounts to about 47%, 84% and 80% decrease respectively when compared to control.

Varying the level of Pb accumulated in the root and shoot of the plants as the concentration of Pb increases from 10 to 30mg/kg, it was observed that the roots and shoots of J. curcas sown in 10mg/kg Pb spiked soil accumulated the highest level of Pb (2.56mg/kg and 0.24mg/kg) which decreased at higher Pb concentrations. High concentration of Pb has been reported to cause damage to roots of plants (Ambika et al., 2016) as the root is the primary target in heavy metal toxicity (Rohan et al., 2010). Since the roots responsible for the absorption of heavy metals from the soil have been affected, lower root and shoot heavy metal accumulation is also expected. This high concentration of Pb at 30mg/kg also reduced the average number of leaves and shoot height of J. curcas sown in Pb contaminated soils (Table 2.0).

The removal efficiency of J. curcas in soils contaminated with chromium of different concentrations (10, 20 and 30mg/kg) was investigated and the results are shown in Figure 2 Table 3. Cr accumulation in the root and shoot of J. curcas in 10, 20 and 30ppm Cr dose increased with respect to control. The root accumulated 0.42, 0.25 and 0.11mg/kg Cr in 10, 20 and 30mg/kg Cr spiked soils which gives a respective increase of 4100%, 2400% and 1000% in the roots when compared to the root of the control (0.01mg/kg). Similarly, the shoot accumulated 0.17mg/kg of Cr in 10mg/kg Pb spiked soil which is about 750% increase when compare to control (0.02mg/kg). At 20 and 30 mg/kg, the shoot of the plant both accumulated 0.16mg/kg Cr which is about 700% higher than the control shoot Cr level. Varying the level of Cr accumulated among the concentrations tested, it was also observed that increasing Cr concentration in the soil reduced Cr uptake by the roots and shoots of the plants. The root and shoot recorded highest Cr level (0.42mg/kg and 0.17mg/kg) at 10mg/kg Cr concentration which reduced at higher Cr concentrations. This result contradicts the report of Ali et al., (2020) where increase in tannery effluent increased the level of Cr in sunflower root, stem, and leaves. Cr has been found to be an essential element needed by the soil and plants for their biochemical roles at low concentrations but could be toxic and causes severe damage to plants at high concentrations (Ranieri et al., 2021). The reduced root growth could be due to inhibition of root cell division and root elongation due to accumulation of chromium in the

vacuoles of the root cells (Datta et al., 2011). This reduced accumulation at higher concentrations could also be a defence mechanism by the plant roots to avoid toxicity to the plant tissues (Rohan et al., 2010). Moreno-Jiménez et al. (2012) reported that one of the defence technique of plant towards Arsenic (As) toxicity is the retention of As in the rhizosphere and this can be achieved in several ways such as the formation of iron plaque through the active release of O_2 from roots in flooded soils or the formation of iron oxide/hydroxides surrounding roots in aerated soils, which adsorbs As and reduce plant uptake.

An effective phytoextraction is determined by the ability of plants to translocate metals to the aerial parts of the plant (Begonia et al., 2005). Within each plant, it was observed that J. curcas sown in10mg/kg spiked soil retained more Pb in their roots than the shoots. Similarly, the translocation of Cr to the shoot of J. curcas in 10 and 20mg/kg Cr spiked soils from the root was less as more Cr accumulated in the roots than the shoots. This demonstrates the lower ability of the plant to translocate Pb and Cr to the aerial parts of the plant and this is a limiting factor in the phytoextraction of metals. Poonam et al., (2018) reported that Pb binds to the carboxylic acid group of mucilage uronic acids on the root's surface and remains stored in the root. Lina et al., (2018) observed a higher concentration of Pb in the root of Cordyline fruicosa (L) than the shoot and attributed it to the low solubility of Pb and low translocation power of Pb from roots to other plant organs. Poonam et al., (2018) observed higher level of Cr in the roots than the shoots of C. procera and R. Communis sown in tannery contaminated soil and attributed it to the immobilization of sulphydryl group (-SH) of soil constituents and Cr complex in the root vacuoles resulting in less translocation of Cr to the upper parts of the plants.

At 20 and 30mg/kg Pb dose, J. curcas translocated more Pb ions to the shoot. More Cr was also observed in the shoot than in the root of the plants in 10 and 20mg/kg Cr spiked soils. This indicated that the absorbed metals were translocated to the aerial parts to reduce the effects of heavy metal toxicity on the root (Rohan et al., 2010). Metal translocation to the shoot of plants is facilitated by the chelation of metals by phytosiderophores, an exudates produced in the root of plants. Phytosiderophore-metal complex moves easily across the cell membranes by preventing metals from binding to the negatively charged cell walls (Liphadzi et al., 2022). This result agrees with that of MacFarlane et al., (2011) who reported lesser accumulation of Pb in the roots than other parts in the accumulation, growth effect, and toxicity of Cu, Pb and Zn in grey mangrove, Avicennia marina (Forsk.).

TABLE 3.0: Concentration (mg/kg) of metals in the root and shoot of J. curcas, BCF and TF

	Pb				Cr			
Amount spiked	Root	Shoot	BCF	TF	Root	Shoot	BCF	TF
10	2.56	0.24	0.28	0.09	0.42	0.17	0.059	0.41
20	0.04	0.07	0.0055	1.75	0.25	0.16	0.021	0.64
30	0.09	0.09	0.006	1.00	0.11	0.16	0.009	1.46
Control	0.53	0.45	61.25	0.849	0.01	0.02	2.069	2.00

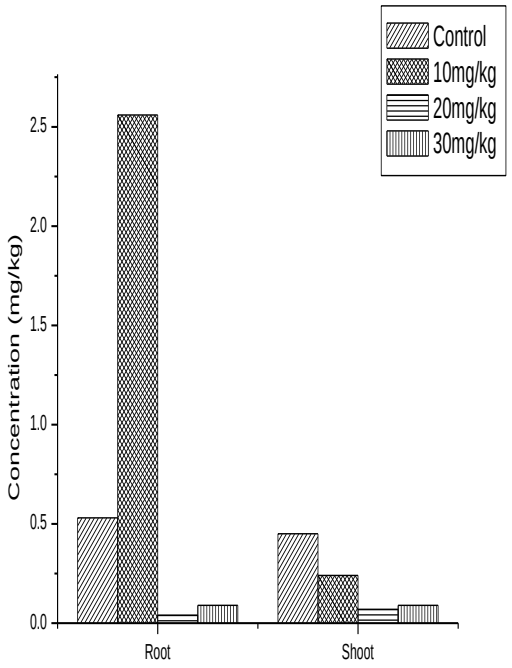


Figure 1: Concentration (mg/kg) of Pb in the root and shoot of J. curcas

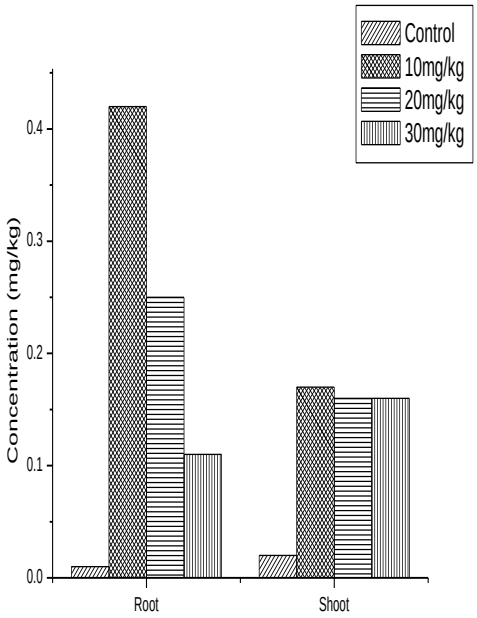


Figure 2: Concentration (mg/kg) of Cr in the root and shoot of J. curcas

D. Bio concentration Factor (BCF)

The ability of plant to concentrate and retain heavy metals in its tissues is very essential as it determines the success of phytoremediation (Aiyesanmi et al., 2021). The relation between metal contents in the different plant parts and its total content in soil was calculated as bio concentration factor (BCF) (Yoon et al., 2006; Cluis., 2004). Blaylock, et al, (1997) categorised accumulation efficiency of plants into three categories depending on their BCF levels; BCF was categorized as; excluder if BCF <1; accumulator if BCF = 1 – 10; hyper accumulator if BCF > 10.

In this study, the BCF of J. curcas for Pb and Cr in the control soils are 61.25 and 2.07 respectively. These values showed that both metals were accumulated effectively by the plant. Using Blaylock, et al, (1997) classification, J. curcas could be classified as a hyper accumulator of Pb and an accumulator of Cr in the control soil. The BCF of J. curcas for Pb and Cr in 10, 20 and 30ppm spiked soils showed that the BCF values of these metals decreased as the metal levels in the soil increased. The BCF values of J. curcas for Pb and Cr followed the order 10mg/kg (0.28) > 20ppm (0.006) = 30ppm (0.006) and 10ppm (0.059) > 20ppm (0.021) > 30ppm (0.009). The result showed that the BCF of the plant reduced with respect to control and also reduced with as the metal concentration in the soil increased from 10 to 30ppm. The reduced BCF of J. curcas for these metals with respect to control could be due to high concentration of these metals in the soil compared to the background concentration of these metals (control). High concentration of these metals in the growth medium could damage the plant roots due to toxicity, hence reduced accumulation of these metals. Using Blaylock, et al, (1997) classification, the accumulation of Pb and Cr by J. curcas in all the spiked soils showed that this plant is an excluder at the tested concentrations.

E. Transfer Factor (TF)

The ability of plants to transport metals from roots to edible parts is characterized by the TF which is defined as the ratio of the metal concentration in the shoots to that in the roots Marchiol et al,. (2004). Plants with TF values > 1 are classified as high-efficiency plants for metal translocation from the roots to shoots, hence, these metals are removed by

phytoextraction process. On the other hand, plants with TF values < 1 indicates low metal translocation, hence, Phytostabilization technique was employed in the removal of the metals from the soil. The results obtained showed that the TF of Pb in 20ppm Pb spiked soil and the TF of Cr in the control soil was greater than 1while the TF of Pb in 30ppm Pb spiked soil was equal to 1 indicating that these metals were translocated to the shoot. It can be concluded that these metals were removed by phytoextraction technology. In all other treatments for both metals, the TF was less than 1indicating that the metals were stabilized in the soil. The TF values of Pb in Pb spiked soils and control followed the order 20ppm (1.75) > 30ppm (1.00) > control (0.85) > 10ppm (0.09) while the TF values of Cr in Cr spiked soils followed the sequence control (2.00) > 30ppm (1.46) > 20ppm (0.64) > 10ppm (0.41).

IV. Conclusion

This study examined the effect of different metal concentrations on average number of leaves and shoot heights of J. curcas. The potentials of J. curcas in the removal of Pb and Cr from soils spiked with different concentrations (10, 20 and 30ppm) of these metals were also examined. The results obtained were compared to the control. The ability of the plant to accumulate metals in its tissues from the soil and translocate them to the aerial parts was examined using BCF and TF. The results obtained showed that the average number of leaves and shoot height of J. curcas decreased as the level of metals increased in the soil. The total Pb and Cr removal by J. curcas in all concentrations examined decreased as the level of the metals increased in the soil. From the BCF values obtained, it can be concluded that J. curcas is a good accumulator of Pb and Cr under natural soil conditions (BCF>1). In all other concentrations examined for Pb and Cr (spiked soils), the BCF < 1 indicating that at high Pb and Cr levels, the plant is a non hyper accumulator but large biomass of J. curcas can compensate for the low accumulation.

V. Acknowledgments

The author wishes to express their profound gratitude to Engineer Yusuf, S. A. for his financial support, the Director, Department of Forestry, Katsina State for the supply of Jatropha seeds, Dr. Musa Bishir, Center for Dry Land Agriculture, Bayero University Kano for the MPAES analysis, Mallam Aminu of the Soil and Water Laboratory (UMYU) for his technical assistance and the technical team of Chemistry Laboratory, (UMYU) for making their laboratory available for this research.

VI. References

Ahalya N., Ramachand rl T.V. and Kanamadi RD., Dec. 2003 Biosorption of Heavy Metal Research Journal of Chemistry and Environment, Vol.7pp4

Ahmadpour, P; Ahmadpour, F;Mahmud, T.M.M; Arifin Abdu, Soleimani, M and Hosseini Tayefeh ,F. 2012. Phytoremediation of heavy metals: A green technology. African Journal of Biotechnology Vol. 11(76), pp. 14036-14043

Aiyesanmi, A. F., Iyalabake O. Ajayi and Afamefuna E. Okoronkwo (2021).Assessing the potential of Ageratum conyzoides for the phytoremediation of lead-polluted soils. IJRES 8, 27-35

Ali G, Srivastava P S, Iqbal M (2000) Influence of Cadmium and Zinc on growth and photosynthesis of Bacopa monniera L. cultivated in vitro. Biol Plant. 43: 599–601.

Ambika Asati, Mohnish Pichhode and Kumar Nikhil (2016). Effect of Heavy Metals on Plants: An Overview. IJAIEM Volume 5, Issue 3.

Azeez, J. O. Olowoboko, T. B. Bada, B. S. Odedina, J. N. & Onasanya, O. O. (2020): Evaluation of soil metal sorption characteristics and heavy metal extractive ability of indigenous plant species in Abeokuta, Nigeria, International Journal of Phytoremediation, DOI: 10.1080/15226514.2020.1717433

Begonia, M. T. Begonia, G. B. Ighoavodha, M. and Gilliard, D. (2005). Lead Accumulation by Tall Fescue (Festuca arundinacea Schreb.) Grown on a Lead-Contaminated Soil. Int. J. Environ. Res. Public Health 2005, 2(2), 228–233

Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., et al. (1997). Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environmental Science & Technology, 31, 860–865

Brooks, R.R., Baker, A.J.M. and Malaisse, F. (1992). The unique flora of the Copper Hills of Zaire. Research and Exploration. 8, 338-351.

Chigbo Chibuike Onyema (2013). Phytoremediation Potential for Co -Contaminated Soils. Ph.D Thesis. University of Birmingham.

Cluis C (2004). Junk-greedy Greens: phytoremediation as a new option for soil decontamination. BioTech J. 2:61-67.

Cokkizgin Alihan and Hatice Cokkizgin (2010). Effects of lead ($PbCl_2$) stress on germination of lentil (Lens culinaris Medic.) lines .African Journal of Biotechnology Vol. 9(50), pp. 8608-8612.

Dagne Bekele Bahiru, (2017). Levels of Some Toxic Heavy Metals in Selected Vegetables, Soil and Wastewater Around Eastern Industry Zone, Central Ethiopia. Msc Thesis Haramaya University, Haramaya.

Danh, L.T., P. Truong, R. Mammucari, T. Tran and N. Foster, 2009. Vetiver grass, Vetiveria zizanioides: A choice plant for phytoremediation of heavy metals and organic wastes. Int. J. Phytorem.,11:664-691.

Datta, J.K., Bandhyopadhyay, A., Banerjee, A. and Mondal, N.K. (2011). Phytotoxic effect of chromium on the germination, seedling growth of some wheat (Triticum aestivum L.) cultivars under laboratory condition. Journal of Agricultural Technology Vol. 7(2): 395-402

Ehsan, N., R. Nawaz, S. Ahmad, M.M. Khan and J. Hayat. (2016). Phytoremediation of chromium contaminated soil by an ornamental plant Vinca (Vinca rosea L.). Journal of Environmental & Agricultural Sciences. 7: 29-34.

Évio Eduardo Chaves de Melo; Clístenes Williams Araújo do Nascimento; Adriana Maria de Aguiar Accioly; Ana Cristiane Queiroz Santos. (2008). Phytoextraction and Fractionation of Heavy Metals In Soil After Multiple Applications of Natural Chelants Sci. Agric. (Piracicaba, Braz.), v.65, n.1, p.61-68,

Helmisaari, H.S., Derome, J., Fritze, H., Nieminen, T., Palmgren, K., Salemaa, M. and Vanha Majamaa, I. (1995). Copper in Scots pine forests around a heavy-metal smelter in South-Western Finland. Water, Air and Soil Pollution. 85, 1727-1732

Jadia CD, Fulekar MH (2009). Phytoremediation of heavy metals: Recent techniques. Afr. J. Biotechnol. 8(6):921-928.

Kisku. G. C., Barman. S. C. and Shargava, S. K. (2000). Contamination of Soil and plants with PTE irrigated with mixed industrial effluent and its impact on the environment. Water, Air and Soil Pollution, 120: 121 – 137.

Li, F., Fan, Z., Xiao, P., Oh, K., X. Ma, and W. Hou, (2009). "Contamination, chemical speciation and vertical distribution of heavy metals in soils of an old and large industrial zone in Northeast China," Environ. Geol. vol. 57, pp. 1815-1823.

Lina herlina, Budi widianarko and Henna rya sunoko. (2018). Phytoremediation of Lead Contaminated Soils using Cordyline fruicosa (L). E 3S Web of Conferences 73 ICENIS 2018 50

Liphadzi, M. S., Kirkham, M. B. (2006). Availability and Plant Uptake of Heavy Metals in EDTA – Assisted Phytoremediation of Soils and Composted Biosolids. South African Journal of Botany 72, 391 – 397.

Macfarlane, G. R. and Burchett, M. D. "Toxicity, Growth and Accumulation Relationships of Copper, Lead and Zinc in the Grey Mangrove Avicennia marina (Forsk.) Vierh," 2011.)

Marchiol, L., Sacco, P., Assolari, S., and Zerbi, G. (2004). Reclamation of polluted soil: phytoremediation potential of crop-related Brassica species. Water Air Soil Pollut., 158: 345–356. 25

Monisha Jaishankar, Tenzin Tseten, Naresh Anbalagan, Blessy B. Mathew , Krishnamurthy N. Beeregowda. (2014). Toxicity, mechanism and Effect of ionic and chelate assisted hexavalent chromium on mung bean seedlings (Vigna Radiata l. health effects of some heavy metals. Interdiscip Toxicol.; Vol. 7(2): 60–72.

Morais S, Costa FG, Pereira ML. (2012). Heavy metals and human health, in Environmental health – emerging issues and practice (Oosthuizen J ed), pp. 227–246.

Moreno-Jiménez, E., Manzano, R., Esteban, E., Peñalosa, J. 2010. The fate of arsenic in soils adjacent to an old mine site (Bustarviejo, Spain): mobility and transfer to native flora. J Soils Sediments 10, 301–312.

Muhammad Shafiq., Iqba, M. Zafar., Mohammad, Athar. (2008) Effect of lead and cadmium on germination and seedling growth of Leucaena leucocephala. J. Appl. Sci. Environ. Manage. Vol. 12(2) 61 – 66.

Muthusaravanan, S., Sivarajasekar, N., Vivek, J. S., Paramasivan T., Mu. Naushad, . Prakashmaran , J., Gayathri, V., Omar K. Al-Duaij. (2018) Phytoremediation of heavy metals: mechanisms, methods and enhancements. Article in Environmental Chemistry Letters · June 2018 DOI: 10.1007/s10311-018-0762-3

Poonam Rani, Adarsh Kumar, Ramesh Chandra Arya, and Tripti, (2018), "Ricinus communis and Calotropis procera As Putative Plant Species for the Phytostabilization of Tannery Contaminated Soil: A Dynamic Approach"in The fourth International Scientific Conference on Ecology and Geography of Plants and Plant Communities, KnE Life Sciences, pages 10–18. DOI 10.18502/kls.v4i7.3214

Prasad M.N.V and Freitas H.M.D.O. 2003. Metal hyperaccumulation in plants-biodiversity prospecting for phytoremediation technology. Electron. J. Biotechnol., 6: 275-321.

Rascio, Nicoletta; Navari-Izzo, Flavia (2011). "Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting?". Plant Science. 180 (2): 169–181.

Rashmi Verma and Pratima Dwivedi (2013). Heavy metal water pollution- A case study Recent Research in Science and Technology, 5(5): 98-99.

Ranieri, E.; D'Onghia, G.; Ranieri, F.; Petrella, A.; Spagnolo, V.; Ranieri, A.C. (2021). Phytoextraction of Cr(VI)-Contaminated Soil by Phyllostachys pubescens: A Case Study. Toxics, 9, 312.

Ravindra K., Gautam, Sanjay K. Sharma., Suresh Mahiyab and Mahesh C. Chattopadhyaya (2014). Contamination of Heavy Metals in Aquatic Media: Transport, Toxicity and Technologies for Remediation. Environmental Chemistry Research Laboratory, Department of Chemistry, University of Allahabad, Allahabad, 211 002, India; Green Chemistry & Sustainability Research Group, Department of Chemistry, JECRC University, Jaipur, 303905, India.

Rohan J. D'Souzaa, Mayank Varuna, Jamson Masihb, Manoj S. Paula.(2010) Identification of Calotropis procera L. as a potential phytoaccumulator of heavy metals from contaminated soils in Urban North Central India. Journal of Hazardous Materials 184, 457–464.

Singh Jiwan and Kalamdhad Ajay S (2011). Effects of Heavy Metals on Soil, Plants, Human Health and Aquatic Life. International Journal of Research in Chemistry and Environment Vol. 1 Issue (15-21)

Sen Zhang, Xia Lu, Yuanzhi Zhang, Gege Nie and Yurong Li. (2019). Estimation of Soil Organic Matter, Total Nitrogen and Total Carbon in Sustainable Coastal Wetlands. Sustainability, 11, 667

United States Department of Agriculture. National Resource Conservation Service (1998). Soil Quality Indicator pH.

Wei S, Teixeira da Silva JA, Zhou Q (2008). Agro-improving method of phytoextracting heavy metal contaminated soil. J. Hazard. Mater. 150(3):662-668.

Wuana, R. A., Okieimen, F. E. (2011). Heavy metals in contaminated soils. A review of sources, chemistry, risk and the best available stategy for remediation. ISRN Ecology International Scholarly Research Network 402647 : 20.

Yao H., Xu J. and Huang C (2003). Substrate utilization pattern, biomass and activity of microbial communities in a sequence of heavy metalpolluted paddy soils. Geoderma, 115, 139– 148.

Yoon J, CaoX, Zhou Q, Ma LQ (2006). Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci. Total Environ. 368(2-3):456-464.

Zarcinas BA, Ishak CF, McLaughlin MJ, Cozens G. (2004). Heavy metals in soils and crops in Southeast Asia. Environ. Geochem. Health 26(3):343-357.

UV-VISIBLE SPECTROPHOTOMETRIC DETERMINATION OF DIETARY IRON IN BEANS, EGG, FISH AND PORK SOLD IN UNIVERSITY OF AGRICULTURE. MAKURDI NIGERIA

***Agada F.O** [1]
Department of Chemistry, J.S Tarka Uiversity Makurdi, Benue State, Nigeria
Itodo A.U PhD [1]
Department of Chemistry, J.S Tarka Uiversity Makurdi, Benue State, Nigeria
Obinna O.O PhD [2]
Department of Chemistry, J.S Tarka Uiversity Makurdi, Benue State, Nigeria
Audu S.S PhD [2]
Department of Science Laboratory Technology, Nasarawa State University, Keffi Nasarawa State
***Shaibu B.**[2]
Department of Zoology, J.S Tarka Uiversity Makurdi, Benue State, Nigeria

ABSTRACT
Determination of dietary iron level in pork, fish, egg and beans samples was carried out using UV-Visible spectrophotometric method after acid digestion. Results from the study revealed that Beans sample presented the highest value of dietary iron with the absorbance value of 0.750 followed by fish with 0.304, followed by egg with 0.263 the least value is pork with 0.256. It was however concluded that all the samples are good sources of iron and recommended as supplement for body iron requirements
Key words; Dietary iron; Iron rich foods, Iron pork; Iron in fish; Iron beans

SEPTICEMIA OF BEES

Ivan PAVLOVIC[1]
[1] Scientific Institute of Veterinary Medicine of Serbia, Belgrade;Serbia
Milan STEVANOVIC[2]
[2]Academy of Beekeeping and Apitherapy of Serbia, Belgrade, Serbia
Nemanja ZDRAVKOVIC[3]
[3] Scientific Institute of Veterinary Medicine of Serbia, Belgrade;Serbia
Narcisa MEDERLE[4]
[4] Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, Timisoara, Romania
Aleksandra TASIC[5]
[5] Scientific Institute of Veterinary Medicine of Serbia, Belgrade; Serbia

ABSTRACT
Septicemia are an infectious disease of bee colonies, which occurs under adverse conditions of detention, is accompanied by the death of adult bees. Diseasee is caused by *Bacterium apisepticus* – polymorphic, gram-negative, small, mobile, spore-forming bacteria. The bacterium is widely distributed in nature.. Bees become infected via their tracheal system. Conditions for of disease include swarming and building honeycomb. It affects drones and worker bees. Usually strikes in May and wears down in October. The development of septicemia is promoted by high humidity: rainy summer, the penetration of precipitation into the nests of bees, the setting of an apiary in low, shaded and waterlogged places, liquid, unsealed food in the winter and a wet winter hut. The forms of the disease can be pronounced, well expressed, under which there is a strong weakening of families, and hidden, when there is a slight death of bees. The major symptoms are a change in the colour of the haemolymph of adult bees from apple brown to chalky white and a rapid degenerationof muscles. As a consequence of the destruction of the connective tissues of the thorax, legs, wings and antennae, bees fall apartwhen handled. Affected bees in colonies appear restless,do not feed and appear to be unable to fly.Dead or dying bees also have a putrid odour The disease itself can last from one to a few days (8-15). The bee mortality rate can vary depending on weather conditions - the weather conditions can lower or even sometimes remove the disease, especially if hygiene measures are implemented and from 10 to 90% of infected bees.

Keywords: septicemia of bees, *Bacterium apisepticus*, epidemiology

AMELIORATIVE EFFECT OF *ALLIUM CEPA* AND *CURCUMA LONGA* POWDER ON LIVER AND KIDNEY FUNCTION INDICES OF MALE ALBINO RATS EXPOSED TO 2,3,7,8-TERACHOLORODIBENZO-P-DIOXIN

U.A. Sabiu[1*]
G.O. Adejo[1]
A.S., Idoko[1]
N. Lawal[1]
Z. S. Abubakar[2]
1 Department of Biochemistry and Molecular Biology, Federal University Dutsin-Ma
2 Department of Biochemistry, Umaru Musa Yaradua University, Katsina

ABSTRACT
Industrial and environmental activities continuously release chemicals such as 2,3,7,8-teracholorodibenzo-p-dioxin (TCDD) that are hazardous to human health. This research seeks to assess the potentials of *Allium cepa* and *Curcuma longa* in suppressing 2,3,7,8-teracholorodibenzo-p-dioxin toxicity. Forty (40) male Wistar rats weighing 121.77±16.23g were divided into eight groups of five (5) rats per group. Normal control was fed standard diet and given 0.5ml dimethyl sulfoxide (DMSO)/kg/week instead of TCDD while the positive control was fed standard diet, and given 2µg/kg/week of TCDD in 0.5ml DMSO. Other groups received 2µg/kg/week of TCDD in 0.5ml DMSO and were fed varied inclusion levels of powdered *Allium cepa* and *Curcuma longa.* On the 35th day of the experimental period, the rats were sacrificed and serum prepared from collected blood for Liver function enzymes analysis, Liver function metabolites, Electrolytes and kidney function metabolites. All treated groups had significantly ($p<0.05$) lower activities of liver enzymes when compared with the negetive control. Other liver function metabolites were significantly ($p<0.05$) higher in the treated groups than the negative control. Electrolytes and other kidney function metabolites were significantly improved upon the inclusion of *Allium cepa* and *Curcuma longa.* Therefore, we conclude that hepatotoxicity and kidney failure due to exposure to dioxin in rats could be suppressed by consumption of 2.5% to 5% inclusion levels of powdered onion and/or turmeric in the diet.
Key words; 2,3,7,8-teracholorodibenzo-p-dioxin; Electrolytes; Liver; Kidney; *Allium cepa*; *Curcuma longa*.

INTRODUCTION
Dioxins organic pollutants belong to the group of persistent organic pollutants (POPs) (Kogevinas, 2011). TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is by-products of waste incineration and chemical manufacturing processes (WHO, 2010; WHO, 2016). The important routes of dioxin entry into the organism include the digestion system, respiratory system and the skin. Dioxins have a high affinity to fats and can dissolve in them; thus, they can be delivered and stored in the organism in a simple way (Sonne *et at*., 2006). Dioxins are transported from the external environment to the adipose tissue or liver through the plasma lipids, and TCDD binds to the aryl hydrocarbon receptor (AhR) in cytosol of hepatocytes (Stevens *et al*., 2009). The active dioxin-receptor complex is translocated to the cell nucleus and binds to the dioxin response element on DNA (dioxin responsive element, DRE). This complex is a signal for transcription of cytochromes P450 and P448 (aryl hydrocarbon hydroxylase – AHH) genes. The highest concentration of AHH, the enzyme responsible for dioxin conversions, occurs in endoplasmic reticulum of hepatocyte. In the initial phase, the

hydrolytic degradation of dioxins by monooxygenase occurs and then the products of this reaction are conjugated with glucuronic acid or the glutathione molecule (GSH). After that, these complexes are released from organisms with urea or bile (Stevens, *et al*., 2009).

The World Health Organization (WHO) defines traditional medicinal plants as natural plant materials which are used at least or in the absence industrial processing for the treatment of diseases at a local or regional scale (Tilburt and Kaptchuk, 2008). The beginning of the development of herbal medicines was concurrent with the development of chemistry and isolation, purification, and determination of plant compounds (Shakya *et al*., 2012). Thus, medicinal plant components such as *Allium cepa* and *Curcoma longa* that suppress AhR transformation are explored in this research for the treatment or prevention of dioxin toxicity in liver and kidney.

MATERIALS AND METHODS

Materials

Feed ingredients

Soya bean meal (SBM), corn oil, palm oil, bone meal, cellulose, salt mix, methionine and pre mix were purchased from Dutsin-Ma central market. Corn starch was prepared from corn after soaking for 48 hours.

Identification of plant materials

Turmeric and onion were collected from Ajiwa farms, Rimi local government area of Katsina State in Nigeria. The plants were identified by a Botanist as *Curcuma longa* and *Allium cepa* with voucher numbers FUDNA/PSB/00143 and FUDMA/PSB/0089 respectively in the Department of Biological Sciences, Federal University Dutsin-Ma Katsina State, Nigeria.

Chemicals

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) was obtained from Loba Chemie PVT Ltd. Assay kits were purchased from trusted suppliers and were products of Randox Ltd.

Plant Preparation

The plants turmeric and onion were dried in an envelope exposed to sunlight, pulverized and sieved using 0.05 mm mesh. The sieved samples were mixed thoroughly in appropriate ratios.

Feed formulation

Standard rodent diet was prepared by approximately mixing corn starch, SBM, cellulose, salt, vitamin mix and mineral mix as stated in Idoko *et al*. (2022) as shown in Table 1.

Table 1: Feed composition breakdown

Feed component	Control diet (g/kg)	Control diet (%)
Corn starch	554.5	55.45
SBM	320	32
Cellulose	45	4.5
Bone Meal	12.5	1.25
Palm oil	60	6
Salt mix	3	0.3
Mineral mix	2.5	0.25
Methionine	2.5	0.25

Mineral mix (g/kg); $CaCO_3$ (15.258), $CoCl_2.H_2O$ (0.001), $ZnCl_2$ (0.001), $CuSO_4.5H_2O$ (0.019), $FeSO_4.7H_2O$ (1.078), $MgSO_4$ (2.929), $MnSO_4.2H_2O$ (0.178), KI (0.032), KH_2PO_4 (15.559) and NaCl (5.573).

Vitamin mix (*g/kg diets*); *thiamine (0.02), riboflavin (0.03), pyridoxine (0.01), p-aminobenzoic acid (0.02), myo-inositol (2.00), biotin (0.001), menadione (0.01), ergocalciferol (0.4), choline-HCL (2.0), and cellulose (3.31), α-tocopherol acetate (50), retinal palmitate (0.4), calcium pantothenate (0.0016) and folic acid (0.0002).*

Formulation of supplemented diet

The diets were supplemented by mixing the formulated rat diet with the prepared plant samples in ratio of 95:5 and 97.5:2.5 to obtain 5% and 2.5% supplementation respectively. Another supplemented diet was formed by mixing both the plant material in the ratio of 95:5 and 97.5:2.5 for 5% and 2.5% respectively.

Experimental design

Forty (40) male Wistar rats averagely weighing 121.77±16.23 g were divided into eight (8) groups of five (5) rats each as follows:

NC: Normal control was fed standard diet and given 0.5 ml DMSO/week.

PC: Positive control was fed with standard diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.

2.5O: Rats fed on 2.5 % onion supplemented diet, and given 2 µg/kg/week of TCDD in 05 ml DMSO.

5O: Rats fed on 5 % onion supplemented diet, and given 2 µg/kg/week of TCDD in 05 ml DMSO.

2.5T: Rats fed on 2.5 % turmeric supplemented diet, and given 2 µg/kg/week of TCDD in 05 ml DMSO.

5T: Rats fed on 5 % turmeric supplemented diet, and given 2 µg/kg/week of TCDD in 05 ml DMSO.

2.5Mix: Rats fed on 2.5 % mixture of onion and turmeric supplemented diet, and given 2 µg/kg/week of TCDD in 05 ml DMSO.

5Mix; Rats fed on 5 % mixture of onion and turmeric supplemented diet, and given 2 µg/kg/week of TCDD in 05 ml DMSO.

The administration of dioxin solution were by gavage in all groups. The rats were all maintained on their respective diets *ad libitum* for a period of five (5) weeks.

Blood sample collection

At the end of the 5 weeks, the rats were weighted, anesthetized and sacrificed by cutting the jugular vein. Serum was obtained from the collected blood samples by centrifugation at 1500 g for 15 minutes and stored at -20 ^{o}C until analysis (Suragani *et al*., 2014).

Determination of kidney function indices

The serum concentration of electrolytes such as sodium, potassium, chloride and bicarbonate were determined using electrolyte analyser while urea and creatinine were determined using standard chemical methods.

Determination of indices of liver function

Serum concentrations of aspartate aminotransferase (AST), alkaline aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, globulins, total and conjugated bilirubin were determined using standard chemical methods.

Statistical analysis

Results were presented as mean ± standard error of mean (SEM) of 3 determinations. Analysis of variance (ANOVA) and Ducan's new multiple range test were performed using SPSS 16.0 software package at 95 % confidence interval.

Results

The results of electrolytes, urea and creatinine as presented in Table 2 shows that the group exposed to TCDD but fed with 5 % mixed spices supplemented diet (5Mix) has significantly ($p<0.05$) least concentrations of chloride, bicarbonate, creatinine and urea compared to the positive and normal control.

Table 2: Serum urea, creatinine and electrolyte concentrations in dioxin exposed rats treated with *Curcuma longa* and/or *Allium cepa*

Groups	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	HCO_3^- (mmol/l)	Urea (mmol/l)	Creatinine (µmol/l)
NC	136±0.33^c	5.53±0.15^b	101.6±0.88^e	16.00±0.58^a	6.50±0.58^c	64.33±1.76^c
PC	133±0.33^a	5.5±0.03^b	93.7±0.88^a	31.0±0.58^e	9.03±0.09^f	80.67±0.88^e
2.5O	135±0.33^b	5.3±0.06^a	98.7±0.33^d	22.33±0.33^c	6.10±0.06^b	59.67±0.88^b
5O	133±0.58^a	5.6±0.11^c	95.7±1.20^b	25.33±0.67^d	6.50±0.23^c	63.00±2.65^b
2.5T	132±0.58^a	5.8±0.06^c	97.7±0.67^c	17.33±0.33^a	6.8±0.12^c	70.33±0.33^d
5T	135±0.58^b	5.2±0.09^a	98.0±0.58^c	19.00±0.58^b	7.17±0.15^d	62.67±0.67^b
2.5Mix	136±0.33^c	6.0±0.03^d	96.0±1.15^b	17.33±0.88^a	7.53±0.03^e	69.00±0.58^d
5Mix	136±1.45^c	5.7±0.07^c	92.3±0.58^a	16.33±1.20^a	5.7±0.06^a	52.00±1.00^a

The results are means of three determinations ± SEM. Values along the same column with the same superscripts are not significantly different ($p>0.05$).

NC is the normal control fed with normal formulated diet and given 0.5ml DMSO/week
PC is the positive control fed with normal formulated diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
2.5O is the group fed with 2.5 % onion supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
5O is the group fed with 5 % onion supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
2.5T is the group fed with 2.5 % turmeric supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
5T is the group fed with 5 % turmeric supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.

2.5Mix is the group fed with 2.5 % mixture of onion and turmeric supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
5Mix is the group fed with 5 % mixture of onion and turmeric supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
Na^+; Sodium, K^+; potassium, Cl^-; Chloride, HCO_3^-; Bicarbonate.

The results of AST, ALT and ALP presented in table 3 shows that the concentrations are higher ($p>0.05$) in the group of the positive control (PC) compared to the normal control and other groups treated with onion and/or turmeric.

Table 3; Serum AST, ALT and ALP concentrations in dioxin exposed rats treated with *Curcuma longa* and/or *Allium cepa.*

Groups	AST(u/l)	ALT (u/l)	ALP (u/l)
NC	4.00 ± 0.58^a	8.00 ± 0.00^a	69.67 ± 0.88^c
PC	21.33 ± 0.88^f	19.33 ± 0.88^c	109.00 ± 1.45^f
2.5O	4.00 ± 0.58^a	8.33 ± 0.33^a	62.33 ± 1.45^b
5O	11.00 ± 0.58^c	12.33 ± 0.33^b	97.00 ± 0.58^e
2.5T	15.00 ± 0.58^e	9.00 ± 0.58^a	82.67 ± 0.88^d
5T	13.00 ± 0.58^d	8.33 ± 0.88^a	72.00 ± 1.52^c
2.5Mix	2.33 ± 0.33^a	8.67 ± 0.33^a	56.33 ± 0.88^a
5Mix	5.33 ± 0.33^b	11.33 ± 0.33^b	96.33 ± 0.88^e

The results are means of three determinations ± SEM. Values along the same column with the same superscripts are not significantly different ($p>0.05$).
NC is the normal control fed with normal formulated diet and given 0.5ml DMSO/week
PC is the positive control fed with normal formulated diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
2.5O is the group fed with 2.5 % onion supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
5O is the group fed with 5 % onion supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
2.5T is the group fed with 2.5 % turmeric supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.

5T is the group fed with 5 % turmeric supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
2.5Mix is the group fed with 2.5 % mixture of onion and turmeric supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
5Mix is the group fed with 5 % mixture of onion and turmeric supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
AST is aspartate aminotransferase; ALT is Alanine aminotransferase; ALP is alkaline phosphatase.
The result of total protein, albumin, globulins, total bilirubin and conjugated bilirubin presented in table 4 shows that the Positive control (PC) had significantly higher ($p>0.05$) concentrations of globulins, total and conjugated bilirubin compared to the normal control and other groups treated with inclusion of *Curcuma longa* and/or *Allium cepa.*

Table 4; Serum total protein, albumin, globulin, albumin globulin ratio, total and conjugated bilirubin concentrations in dioxin exposed rats treated with *Curcuma longa* and/or *Allium cepa.*

Groups	Tot. protein (g/l)	Albumin (g/l)	Globulin (g/l)	Albumin/Globulin ratio	Tot. Bil. (mg/dl)	Con. Bil. (mg/dl)
NC	58.00±0.58^{b}	23.33±0.88^{b}	34.67±0.88^{cd}	0.72±0.04^{a}	1.43±0.03^{a}	0.23±0.03^{a}
PC	57.66±1.20^{b}	20.33±2.03^{a}	37.33±2.67^{e}	0.56±0.10^{a}	2.83±0.07^{c}	0.67±0.03^{d}
2.5O	61.33±0.67^{c}	32.67±0.33^{de}	28.67±0.33^{a}	1.14±0.002^{c}	1.30±0.06^{a}	0.27±0.03^{a}
5O	52.00±0.58^{a}	24.00±0.58^{b}	28.00±0.58^{ab}	0.86±0.03^{b}	2.20±0.35^{b}	0.53±0.03^{bc}
2.5T	68.00±0.58^{e}	35.33±0.3^{e}	32.67±0.33^{bc}	1.08±0.01^{c}	2.10±0.06^{b}	0.60±0.06^{c}
5T	56.33±0.88^{b}	20.33±0.88^{a}	36.00±0.00^{cd}	0.56±0.02^{a}	2.27±0.09^{b}	0.50±0.06^{bc}
2.5Mix	65.00±0.58^{d}	31.00±0.58^{d}	34.00±1.00^{bcd}	0.72±0.04^{b}	1.27±0.07^{a}	0.23±0.03^{a}
5Mix	58.00±0.58^{b}	27.00±0.58^{c}	31.00±00^{a}	0.87±0.02^{b}	1.90±0.06^{b}	0.40±0.06^{b}

The results are means of three determinations ± SEM. Values along the same column with the same superscripts are not significantly different ($p>0.05$).
NC is the normal control fed with normal formulated diet and given 0.5ml DMSO/week
PC is the positive control fed with normal formulated diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
2.5O is the group fed with 2.5 % onion supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.

5O is the group fed with 5 % onion supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
2.5T is the group fed with 2.5 % turmeric supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
5T is the group fed with 5 % turmeric supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
2.5Mix is the group fed with 2.5 % mixture of onion and turmeric supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.
5Mix is the group fed with 5 % mixture of onion and turmeric supplemented diet and given 2 µg/kg/week of TCDD in 05 ml DMSO.

Tot. protein is total protein; tot. bil. is total bilirubin; con. bil. is conjugated bilirubin.

Discussion

Table 2 shows a significant difference in serum urea, creatinine and some electrolyte concentrations between the test groups and control groups. The kidney is highly susceptible to toxicants because of a high volume of blood flow through it, and it filters large amounts of toxins which can concentrate in the kidney tubules (Lawal *et al.*, 2015). Nephrotoxicity can result in systemic toxicity causing decreased ability to excrete body wastes, inability to maintain body fluids, electrolytes balance and decreased synthesis of essential hormones. Therefore, the measurement of the levels of electrolytes, creatinine and urea plays important roles in determining the synthetic and excretory roles of the kidney (Onukogu, *et al.*, 2019). Creatinine is the catabolic products of creatinine phosphate which is used by the skeletal muscle. It is a metabolite of muscle – creatinine, whose amount in serum is proportional to the body's muscle mass. The amount of creatinine is usually constant, it is easily excreted by the kidneys, and thus elevated levels indicate diminished renal function (Umar *et al.*, 2019). The significant ($p<0.05$) and dose-dependent decrease in serum creatinine following the administration of the supplemented diet indicates a compromised renal functional capacity. The supplementation might have either interfered with creatinine metabolism leading to decreased synthesis or might have compromised tubular excretion (Yusuf *et al.*, 2018). Urea is an end product of protein metabolism and its product reflects diet protein intake and protein catabolic rate. It is a waste product that is left over from the breakdown of protein. Urea circulates in the blood until it is filtered out by the kidneys and excreted in the urine (Latha *et al*., 2016). If the kidneys are not functioning properly, there will be excess urea levels in the bloodstream. Impaired balance of nitrogen coupled with lowered protein synthesis leads to increased concentration of urea and creatinine in serum which indicates progressive renal damage. The significant alteration in serum urea and creatinine following the administration of the onion and turmeric may be due to decreased protein catabolism or renal dysfunction (Lawal *et al*., 2015). The non-significant effects in the level parts of the electrolytes such as chloride and bicarbonate following administrations of supplemented diets suggests that normal functioning of kidney tubules as regard to these electrolytes was preserved (Umar *et al*., 2019). However, serum potassium and sodium concentrations in albino rats following supplementation significantly ($p<0.05$) increase when compared with the control rats. These findings suggest that some aspects of tubular functioning as it relates to potassium and sodium have been compromised (Yakubu *et al*., 2003).

TCDD induces hepatocyte toxicity and mitochondrial dysfunction by mechanism involving generation of ROS (Salim *et al.*, 2013). However, the groups treated with 2.5% and 5% *Curcuma longa* and/ *Allium cepa* shows various degree of improvements in the liver functions, with the groups treated with inclusion of mixed *Curcuma longa* and *Allium cepa* supplemented diets showing better improvements ($p>0.05$) in liver functions, indicating an

improvement on the functions of the hepatocytes (Salim *et al.*, 2013). Cerny *et al*., (2011) reported that the liver protection was clearly demonstrated by significant decrease in serum aminotransferases (AST, ALT) after *Curcuma longa* and *Allium cepa* are administered to the rats. The *Curcuma longa* and *Allium cepa*'s hepatoprotective effect is mainly a result of its antioxidant properties, as well as its ability to decrease the formation of pro-inflammatory cytokines, free radical scavenging, ROS scavenging ability and cell proliferation activity in cell line (Suhit *et al*., 2010). It was noted that rats treated with *Curcuma1 longa* and *Allium cepa* have enzymatic activities lower than those not given the treatment. This means that *Curcuma longa* and *Allium cepa* are effective against any inflammatory condition that can affect the liver. These anti-inflammatory properties may be attributed to their ability to inhibit both biosynthesis of inflammatory prostaglandins from arachidonic acid and neutrophil function during inflammatory states (Tayyem *et al*., 2006; Hanai and Sugimoto, 2009). The result of this study showed that the application of turmeric and onion markedly inhibited acute hepatic failure in a rat model, which was induced at least in part by free radical formation. The results suggest that turmeric and its active component curcumin may be effective against acute liver stress by enhancing redox capacity and antioxidant enzyme activity. Acute dioxin administration is a widely used experimental model that mimics the acute liver failure caused by toxic substances (Ma *et al.,* 2015). Although the liver plays a key role in biotransforming and detoxification of chemicals, certain medicinal agents, when taken in excess, may injure the organ. Other chemical agents or industrial agents can also induce hepatotoxicity. These chemicals often cause subclinical injury to the liver, increasing liver enzymes, but not causing pathological abnormalities such as histological and hepatosteatosis status changes. Curcumin and onion also control the out flow of ALT, AST and ALP from the liver cells. A similar pattern was reported recently in cases of acute hepatic and transient stress (Wang *et al.,* 2020). AST is localized in the mitochondria, whereas ALT is distributed throughout the cytoplasm. In the case of hepatic stress, mitochondrial damage with ROS accumulation tends to increase the level of AST rather than ALT (Sheng *et al.,* 2015). The manifestation of acute hepatotoxicity in acute stress is highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure (Jiao *et al.,* 2015).

Conclusion

Many harmful effects of TCDD were observed in this study, we can therefore conclude that, supplementation of diets with *Curcuma longa* and/or *Allium cepa* could potentially improve renal functions and prevent hepatic damage in animal models exposed to TCDD.

References

Černý, D., Lekić, N., Váňová, K., Muchová, L., Hořínek, A., Kmoníčková, E., and Farghali, H. (2011). Hepatoprotective effect of curcumin in lipopolysaccharide/-galactosamine model of liver injury in rats: relationship to HO-1/CO antioxidant system. *Fitoterapia*, *82*(5), 786-791.

Hanai, H., and Sugimoto, K. (2009). Curcumin has bright prospects for the treatment of inflammatory bowel disease. *Current pharmaceutical design*, *15*(18), 2087-2094.

Idoko, A.S., Abdullahi, A.B., Maibalangu, M., Nura, L., Imam, N.U., Bonomi, Z.M., Muhammed, F. and Umar, S. (2022). Allium sativum and Curcuma longa Powder Protect Against Hepatotoxic and Nephrotoxic effect of High Fructose Diet. *FUOYE Journal of Pure and Applied Science,* 7(1):59-78.

Jiao J, Friedman SL, Aloman C. Hepatic fbrosis. Curr Opin Gastroenterol. 2009;25(3):223–9.

Kogevinas, M. (2011). Epidemiological approaches in the investigation of environmental causes of cancer: the case of dioxins and water disinfection by-products. *Environ Health.*, 10: 3.

Latha, S., VijayaKumar, R., Senthil Kumar, B. R., Bupesh, G., and Kumar, T. S. V. (2016). Acute and Repeated Oral Toxicity of Antidiabetic Polyherbal Formulation Flax Seed, Fenugreek and Jamun Seeds in Wistar Albino Rat. *J Diabetes Metab*, *7*(656), 2.

Lawal, B., Shittu, O. K., Abubakar, A. N., Umar, M. B., Ibrahim, A. M., and Haruna, G. M. (2015). Biochemical evaluation in Wister rats (Rattus novergicus) following chronic exposure of methanol leaf extract of Telfairia occcidentalis. *J Pharm Biomed Sci*, *5*(9), 740-744.

Lawal, B., Shittu, O. K., Busari, M. B., Sani, S., and Aisha, M. I. (2015). Safety evaluation of giant African land snails (Archachatina marginata) haemolymph on hematological and biochemical parameters of albino rats. *J Adv Med Pharm Sci*, *3*(3), 122-30.

Ma, J.Q., Li, Z., Xie, W.R., Liu, C.M. and Liu, S.S. (2015) Quercetin protects mouse liver against CCl(4)-induced inflammation by the TLR2/4 and MAPK/NF-kappaB pathway. *Int Immunopharmacol*;28(1):531–9

Onukogu, S. C., Tsado, A. N., Muhammad, F. M., Alawode, R. A., Suleiman, A., and Ojo, O. P. (2019). In vitro antioxidants, antimicrobials and biochemical response of methanol leaf extract of eucalyptus camaldulensis following sub-acute administration to rats. *Saudi J Biomed Res*, *4*(11), 405-11.

Salim, L. A., Mohan, S., Othman, R., Abdelwahab, S. I., Kamalidehghan, B., Sheikh, B. Y., and Ibrahim, M. Y. (2013). Thymoquinone induces mitochondria-mediated apoptosis in acute lymphoblastic leukaemia in vitro. *Molecules*, *18*(9), 11219-11240.

Shakya, A.K., Sharma, N., Saxena, M., Shrivastava, S., Shukla, S. (2012). Evaluation of the antioxidant and hepatoprotective effect of Majoon-e-Dabeed-ul-ward against carbon tetrachloride induced liver injury. *Exp Toxicol Pathol*, 64:767- 73.

Sheng, J., Zhang, J., Wen, J., Ming, Q., Zhang, J. and Xu, Y. (2015). Correlation of serum alanine aminotransferase and aspartate aminotransferase with coronary heart disease. *Int J Clin Exp Med* ;8(3):4399–404

Sonne, C., Dietz, R., Leifsson, P.S., Born, E.W., Kirkegaard, M., Letcher, R.J. (2006) Are organohalogen contaminants a cofactor in the development of renal lesions in east Greenland polar bears Environ *Toxicol Chem*, 25: 1551–1557.

Stevens, E.A., Mezrich, J.D. and Bradfield, C.A. (2009). The aryl hydrocarbon receptor: a perspective on potential roles in the immune system. Immunology. 127: 299–311.

Suhit, G., Kanitkar, M., Bhonde, R., and Paradkar, A. (2010). Activity of water-soluble turmeric extract using hydrophilic excipients. *LWT-Food Science and Technology*, *43*(1), 59-66.

Suragani, R.N., Cadena, S.M., Cawley, S,M., Sako, D, Mitchel, D., Li, R. and Kumar, R. (2014). Transforming growth factor-β superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis. *Nature medicine*, 20(4):408-414.

Tilburt, J.C. and Kaptchuk, T.J. (2008). Herbal medicine research and global health: an ethical analysis. *Bull World Health Organ*, 86:594-9.

Umar, S. I., Lawal, B., Mohammed, B. A., Obiekezie, C. I., Adewuyi, A. H., Babalola, S. B., and Ariyeloye, S. D. (2019). Antioxidant and Antimicrobial Activities of Naturally Occurring Flavonoids from M. heterophylla and the Safety Evaluation in Wistar Rats. *Iranian Journal of Toxicology*, *13*(4), 39-44

Umar, S. I., Ndako, M., Jigam, A. A., Adefolalu, S. F., Ibikunle, G. F., and Lawal, B. (2019). Antiplasmodial, Anti-inflammatory, anti-nociceptive and safety profile of Maytenus senegalensis root bark extract on hepato-renal integrity in experimental animals. *Comparative Clinical Pathology*, *28*(6), 1571-1579.

Wang, X.Y., Luo, J.P., Chen, R., Zha, X.Q. (2020). Wang, H. The effects of daily supplementation of *Dendrobium huoshanense* polysaccharide on ethanol-induced subacute liver injury in mice by proteomic analysis. Food Function. 5(9):20–35.

World Health Organization (2010). Preventive disease through healthy environments. Exposure to dioxins and dioxin-like substances. http:// www.who.int/ipcs/features/dioxins.pdf?ua=1. Accessed 14 Nov 2017.

World Health Organization (2016). Global Report on Diabetes: World Health Organization; Report No.: 9789241565257; WHO: Geneva, Switzerland.

Yakubu, M. T., Bilbis, L. S., Lawal, M., and Akanji, M. A. (2003). Effect of repeated administration of sildenafil citrate on selected enzyme activities of liver and kidney of male albino rats. *Nig J Pure & Appl Sci*, *18*, 1395-400.

Yusuf, A. A., Lawal, B., Abubakar, A. N., Berinyuy, E. B., Omonije, Y. O., Umar, S. I., and Alhaji, Y. M. (2018). In-vitro antioxidants, antimicrobial and toxicological evaluation of Nigerian Zingiber officinale. *Clinical Phytoscience*, *4*(1), 12:1-8.

Tayyem, R. F., Heath, D. D., Al-Delaimy, W. K., and Rock, C. L. (2006). Curcumin content of turmeric and curry powders. *Nutrition and cancer*, *55*(2), 126-131.

SERUM GLUCOSE LEVELS, LIVER AND KIDNEY FUNCTION INDICES IN ALLOXAN INDUCED DIABETIC RATS SUPPLEMENTED WITH AQUEOUS EXTRACT OF *HELIXANTHERA PARASITICA* LEAVES

Usman, A.*
Abubakar, M.
Department of Biochemistry, Umaru MusaYaradua University, P.M.B 2218, Katsina

ABSTRACT
Diabetes is a metabolic disease typified by elevated levels of blood sugar, resulting to serious injury to the kidneys, blood vessels, eyes, heart and nerves. This research work investigates some biochemical parameters in the liver and kidney of alloxan-induced diabetic Wistar rats treated with aqueous leaf extract of *Helixanthera parasitica*. The extract (200mg/kg) was orally administered to alloxan-induced diabetic Wistar rats (~150mg/kg) for twenty one days, after which some biochemical indices in the serum, liver and kidney were measured and compared with the control. The results shows that serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum urea, direct bilirubin and creatinine of untreated diabetic group were significantly higher ($p<0.05$) when compared with the extract treated group and normal control group. These findings indicated that administration of aqueous leaf extract of *Helixanthera parasitica* to diabetic rats ameliorates some of the adverse effects of diabetes complications.
Key Words: Diabetes, *Helixanthera parasitica*, Liver Functions, Kidney Functions.

ISOLATION OF MULTIDRUG RESISTANT BACTERIA FROM TWO LOCALLY PRODUCED DRINKS SOLD IN NORTH CENTRAL, NIGERIA

Oyedum, U.M.*
Agbala, J.

Department of Microbiology, School of Life Sciences, Federal University of Technology, Minna

ABSTRACT

The predominance of multidrug resistant (MDR) bacteria among the populace, edible foods and drinks is fast becoming the major concern in most communities. Six (6) locally prepared drinks from three locations were aseptically collected and transported to the Microbiology Laboratory of Federal University of Technology, Minna. Samples were serially diluted and were inoculated on various media through the spread plate method. The bacterial isolates were identified based on their Gram reaction and other biochemical tests. The antibiotic susceptibility tests were carried out for the bacterial isolates using the disc diffusion method on Muller Hinton agar. The result revealed that out of all the locally prepared drinks sampled tiger-nut drink (Kunuaya) (3.9×10^3) and Zobo (3.4×10^3) from Federal University of Technology, Minna, Bosso campus had the highest microbial count. Various bacterial pathogens were isolated and identified with *Salmonella* sp having the highest frequency of occurrence (30.7%) and *Klebsiella* sp having the lowest frequency (7.7%). The antibiotic susceptibility tests revealed that all bacterial isolates were Multidrug resistant and as such are a great threat to the health of the general public especially the regular consumers of these locally prepared drinks. Hence, there is a need for adequate and continuous surveillance by food regulatory bodies in Nigeria, to curtail the spread and infections associated with Multidrug resistant bacteria.

Keywords: Locally prepared drinks; Bacteria; Multi-drug resistant bacteria; Zobo; Kunuaya

Introduction

Locally produced drinks are liquids mainly processed from animal or plant sources. They may be regarded as stimulants such as tea, as refreshers such as soft drinks, juices or as nutritional drinks such as milk. The processing of locally produced drinks could either be by simple non-microbial processes or physical techniques (such as malting, boiling, pasteurization and distillation) or may involve microbial process such as fermentation and/or enzyme clarification (Koketso *et al*., 2018; Onuoha & Fatokun, 2014; Umar *et al.*, 2014).

Fermentation by microorganisms, mainly involves the breakdown of sugars to yield acids and then the acids are converted to alcohol. Fermentation is the major processing technique employed in the preparation of over 90% of the diverse locally produced drinks across Africa (Umar *et al.*, 2014). The fermenters and saccharifying enzymes are usually intrinsic to the grains and other ingredients (Koketso *et al*., 2018; Umar *et al.*, 2014).

Locally produced drinks could be classified as either alcoholic (such as burukutu and pito) or non alcoholic (such as kunuaya (tiger-nut milk), kunu-samiya, kunu-zaki, zobo and palm wine) and based on the process involved they could either be regarded as industrially processed beverages or traditionally processed beverages (Kigigha *et al*., 2018).

Locally produced drinks are usually known for their nutritional and therapeutic benefits (Onuoha & Fatokun, 2014), they are basically rich in Vitamins, Minerals and carbohydrates (Umar *et al*., 2014). The additional supplements such as Nuts, spices, Tubers, have

tremendously boosted the protein content and antioxidants properties of most consumed locally produced drinks (Kigigha *et al*., 2018).

Most of these indigenous drinks are usually exposed to certain pathogenic microbes "especially the resistant strains" (Kigigha *et al*., 2018) during the production and packaging of these products. Based on the fact that, these pathogenic organisms are usually associated with the spoilage of the drinks and food borne diseases which lead to severe diseases and deaths, there is need to continuously examine the resistant microbial burden associated with most food and locally produced drinks commonly consumed. Thus this study is therefore said to determine the multi drug resistant (MDR) bacteria associated with locally prepared drinks that are commonly sold in Minna.

MATERIALS AND METHODS

Study Area

The study area was Minna, Niger State. The state is located in the North Central geopolitical zone of Nigeria and covers a landmass of 76,363 square kilometers. It lies between latitude 8^{o}.00-11^{o} .30'N and Longitude 4^{o}.00-8^{o}.00'E (Kigigha *et al*., 2017).

Sample Collection

A total of six samples for each drink namely: kunuaya and zobo were purchased from three different vendors in three different locations (namely: Bosso market, Federal University of Technology Minna, Bosso campus and El-waziri) in Minna, North central region of Nigeria. The samples were taken to the Microbiology laboratory, for further analysis.

Microbiological Analysis of Sample

Serial dilution of the each drink sample was carried out by suspending one mililitre of the kunuaya and zobo samples into 9mL of sterile distilled water in the test tube. The mixture was shaken thoroughly to ensure proper dissolution of the sample. Spread plate method was employed to inoculate the media. Aliquot of 1mL of the sample was pipetted each from 10^{-4} dilution tubes into well labeled Petri dishes containing a 20mL of molten nutrient agar (this was done in triplicate) and was swirled gently to allow proper mixing. The Petri dishes were later incubated at 37°C for 24hrs. The colonies formed were counted and expressed as colony forming unit per millilitre (cfu/ml). The colonies that grow on the growth media that are different in size, shape and color were picked and sub-cultured on MacConkey agar and SSA (*Salmonella Shigella* agar) to determine the cultural characteristics of the organisms. The pure isolates were preserved on agar slant bottle for further investigations.

Identification of Bacteria

The isolated bacteria were identified via Gram staining and other conventional biochemical tests such as: Coagulase, Oxidase, Catalase, Citrate, Urease, Indole and Triple sugar test as described by Cheesbrough, (2010).

Antibiotic Sensitivity Test

The isolates were screened for antimicrobial susceptibility using the Kirby-Bauer agar disc diffusion method .The colony of each organism was transferred into sterile Mueller-Hilton broth and incubated at 37°C for 24hrs.The overnight culture was adjusted to the turbidity equivalent to 0.5 Mcfarland standard by adding 0.85% sterile normal saline to the overnight culture. The adjusted innocula was subcultured on the surface of Mueller-Hilton agar (MHA) and the antibiotic discs such as: Penicillin G (10µg), Augmentin (30µg), Streptomycin(10µg), Ciprofloxacin(5µg), Nalidixic acid(30µg), Gentamycin (10µg), Ofloxacin (5µg), Chloramphenicol(10µg) and so on (Spencer *et al.*, 2014), were aseptically placed at the center of the MHA plate and incubated at 37°C for 24hours. The zones of inhibition of the bacterial isolates were measured using a transparent ruler as described by Clinical and Laboratory Standard Institute (CLSI), (2016).

Results

Out of all the locally prepared drinks sampled, Kunuaya (3.9×10^3) and Zobo (3.4×10^3) drinks obtained from Federal University of Technology Minna, Bosso Campus had the highest microbial count as seen in Table 1.

Table 1: Microbial count of 2 locally prepared drinks from 3 locations in Bosso Minna.

Locations	Sample	Point A	Point B	Point C
El-Waziri	Kunu aya	2.0×10^3	2.9×10^3	3.0×10^3
	Zobo	3.0×10^3	2.8×10^3	2.5×10^3
Federal University of Technology,Minna, Bosso campus	Kunu aya	3.9×10^3	3.0×10^3	3.1×10^3
	Zobo	3.4×10^3	2.9×10^3	3.0×10^3
Bosso market	Kunu aya	2.9×10^3	2.7×10^3	2.6×10^3
	Zobo	2.7×10^3	2.1×10^3	2.8×10^3

Table 2: Biochemical characteristics of the isolated bacteria

Code	GR	Sh	Coa	Cit	Ure	Oxi	Ct	MR	VP	H2S	Ind	Starch	Suspected Organisms
1	+	C	+	+	+	-	+	+	-	-	-	-	*Staphylococcus* sp
2	-	R	-	+	-	+	+	-	+	NA	+	+	*Pseudomonas* sp
3	+	R	-	+	-	-	+	-	+	-	-	+	*Bacillus* sp
4	+	C	+	+	+	-	+	+	-	-	-	-	*Staphylococcus* sp
5	-	R	-	+	+	-	+	-	+	-	-	+	*Klebsiella* sp
6	-	R	-	-	-	-	+	+	-	+	+	-	*Escherichia coli*
7	-	R	-	-	-	-	+	-	+	+	-	-	*Salmonella* sp
8	+	C	+	+	+	-	+	+	-	-	-	-	*Staphylococcus* sp
9	+	R	-	+	-	-	+	-	+	-	-	+	*Bacillus* sp
10	-	R	-	-	-	-	+	-	+	+	-	-	*Salmonella* sp
11	-	R	-	-	-	-	+	+	-	+	+	-	*Escherichia coli*
12	-	R	-	-	-	-	+	-	+	+	-	-	*Salmonella* sp
13	-	R	-	-	-	-	+	-	+	+	-	-	*Salmonella* sp

Key: Isc (Isolate code), GR (Gram Reaction), Sh (Shape), Ct (catalase), Cit (Citrate), H2S (Hydrogen Sulphide), MR (Methyl Red), VP(Voges Proskauer),Ure (Urease), Oxi (Oxidase), Ind (Indole), Glu (Glucose), Coa (Coagulase), C (Cocci), R (Rod), + (Positive), - (Negative) , NA (Not applicable).

Six (6) bacterial isolates were obtained with *Salmonella sp* having the highest frequency of occurrence of 30.7% while *Pseudomonas aeruginosa* and *Klebsiella sp* had the least frequency of occurrence of 7.7% respectively (as seen in Table 3).

Table 3: Frequency of occurrence of bacterial isolate

Organisms	Frequency of occurrence	Percentage of occurrence
Staphylococcus sp	3	23.1
Salmonella sp	4	30.7
Pseudomonas sp	1	7.7
Escherichia coli	2	15.4
Bacillus sp	2	15.4
Klebsiella sp	1	7.7
Total	13	100

Eight (8) bacterial isolates out of thirteen (13) bacteria isolates were obtained from kunuaya, while five (5) bacteria isolates were obtained from Zobo. Samples from Elwaziri area had the highest bacterial contamination with 6 bacterial isolates followed by Samples from FUT Minna, Bosso campus with 4 bacterial isolates and then samples from Bosso Market with 3 bacterial isolates (as seen in Table 4).

Table 4: Sources and location of the bacterial isolates

Bacterial isolates	Number of isolates	Sources of isolates	Location
Staphylococcus sp	3	Kunuaya (2 isolates) Zobo(1 isolate)	El-waziri Bosso campus
Salmonella sp	4	Kunuaya (2 isolates) Zobo(2isolate)	Bosso market El-waziri
Pseudomonas sp	1	Zobo	Bosso campus
Escherichia coli	2	Kunuaya (2 isolates)	Bosso campus El-waziri
Bacillus sp	2	Kunuaya (1 isolates) Zobo(1 isolate)	Bosso market El-waziri
Klebsiella sp	1	Kunuaya	Bosso campus

Table 5.1: Susceptibility profile for Gram positive bacteria

Bacterial isolate	Number of isolate	Z(%)	AMP(%)	R(%)	PEF(%)	CN(%)	CPX(%)	APX(%)	SXT(%)	E(%)	S(%)
Staphylococcus aureus											
S	3	0(0)	0(0)	0(0)	1(33.3)	0(0)	0(0)	0(0)	1(33.3)	0(0)	0(0)
I		0(0)	0(0)	1(33.3)	0(0)	0(0)	0(0)	0(0)	2(66.6)	0(0)	0(0)
R		3(100)	3(100)	2(66.6)	2(66.6)	3(100)	3(100)	3(100)	0(0)	3(100)	3(100)
Bacillus sp											
S	2	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	0(0)	0(0)
I		0(0)	0(0)	0(0)	0(0)	1(50)	0(0)	0(0)	1(50)	0(0)	0(0)
R		2(100)	2(100)	2(100)	2(100)	1(50)	2(100)	2(100)	0(0)	2(100)	2(100)

Key: R= resistance, S=susceptible, I=intermediate, CN=Gentamycin, CPX=Ciprofloxacin, APX=Ampliclox, SXT=Septrin, PEF=Perfloxacin, R=Rifampicin, AMP=Ampicillin, E=Ethromycin,S=Streptomycin,Z=Zithromax

Table 5.2: Susceptibility profile for Gram negative bacteria

Bacterial isolate	Number of isolate	SP(%)	PEF(%)	OFX(%)	CPX(%)	CH(%)	S(%)	AU(%)	CN(%)	AM%)	SXT(%)
Pseudomonas sp											
S	1	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
I		0(0)	1(100)	(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	(0)
R		1(100)	0(0)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
Escherichia coli											
S	2	0(0)	0 (0)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
I		0(0)	1(50)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)
R		2(100)	1(50)	0(0)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	1(50)
Klebsiella sp											
S	1	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
I		0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	1(100)
R		1(100)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)	0(0)
Salmonella sp											
S	4	0(0)	0(0)	1(25)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
I		0(0)	0(0)	0(0)	0(0)	1(25)	0(0)	0(0)	0(0)	0(0)	2(50)
R		4(100)	4(100)	3(75)	4(100)	2(75)	4(100)	4(100)	4(100)	4(100)	2(50)

Key: R= resistance, S=susceptible, I=intermediate, CN=Gentamycin, CPX=Ciprofloxacin, CH=Chloramphenicol, SXT=Septrin, PEF=Perfloxacin,OFX=Ofloxacin, AM=Amoxacillin, SP=Sparfloxacin,S=Streptomycin,AU=Augmentin

DISCUSSION

This study reveal that kunuaya (3.9×10^3) and zobo (3.4×10^3) from F.U.T Minna, Bosso campus are highly contaminated with disease causing microorganisms, as seen in Table 1 . This could be based on the unhygienic packaging associated with most of these drinks, which introduces high microbial contaminants to the drinks before they are consumed. Similarly poor or inadequate use of personal protective wears by the manufacturers of these drinks exposes these locally prepared drinks to high microbial contamination. This result agrees with the findings of (Adesakin and Obiekezie, 2020; Ayandele, 2015) who revealed that improper use of protective wears by manufacturers enhances the introduction of microbial contaminants into the drinks.

The study also revealed that *Salmonella sp.* had the highest frequency of occurrence (30.7%) followed by *Staphylococcus aureus* (23.1%). This could be due to the fact that certain production materials of these locally prepared drinks such as water usually harbor large populations of faecal coliforms (from either human or animal sources) and environmental wastes. This finding agrees with (Musa *et al*., 2018) who revealed that most locally prepared drinks analysed, were highly contaminated by faecal coliforms.

The high bacterial contamination recorded in kununaya compared to zobo drink (as seen in Table 4) could be based on the fact that the raw materials for kunuaya, which are tiger nuts are easily prone to microbial contamination during their growth and harvest in the fields and this usually exposes the tiger nut milk (kunuaya) to heavy contamination with various microbes. Similarly milling machine used to mill the tiger nuts are usually for commercial purpose and in most cases are usually unclean and heavily contaminated with bacteria, which in turn contaminates the tiger nut milk (kunuaya) after the tiger nuts have been milled. However the high contamination of various bacteria observed in locally prepared drinks obtained in Elwaziri area (as seen in Table 4) could be attributed to certain factors such as low personal hygiene of the populace in that area and improper disposal of waste by the populace in that area. This agrees with the finding of (Ayandele, 2015) who revealed that most raw materials used for local drinks are edible roots of crops and hence are prone to microbial contamination.

This study revealed that all bacterial isolates (namely the Gram positive and Gram negative) were all multidrug resistant (Table 5 and 6). This could be attributed to the fact that most bacterial prevalent among the populace or within the study area exhibited multidrug resistant due to the rapid dissemination of the resistant gene's through various genetic transfer material such as plasmids.

Conclusion and Recommendation

This study revealed that two commonly consumed local drinks, namely; kunuaya (tiger nut milk) and zobo are highly contaminated with various bacteria such as: *Staphylococcus sp*, *Salmonella sp*, *Pseudomonas sp*, *Escherichia coli*, *Bacillus sp* and *Klebsiella sp*. However all these bacterial contaminants are multi drug resistant, thus there is an eminent need for Government and food monitoring agencies to enlighten and encourage producers and vendors of these locally prepared drinks, on the importance to employ adequate hygienic standards in the production and packaging of these locally prepared drinks to ensure that bacterial contaminants, especially multi drug resistant bacteria are curtailed and controlled.

REFERENCE

Adesakin, F.O., & Obiekezie, S.O. (2020). Bacteriological and nutritional assessment of tiger nut milk (kunun-aya) consumed by students of Nasarawa State University, Keffi Nigeria. *World Journal of Advanced Research and Reviews*, 6(3), 59-68.

Ayandele,A.A. (2015). Review on tiger nut milk. *Journal of Environmental Science, Toxicology and Food Technology*, Volume 9, (10), 52-56.

Cheesbrough, M. (2010). District Laboratory Practicein Tropical Countries, Part 2, 2nd Edition update. United Kingdom: Cambridge University Press, Cambridge, Pp 107-114.

Clinical and laboratory standards institute (2016). Performance standards for antimicrobial susceptibility testing.26th Edition. Supplement M100S. CLSI, Wayne,Pages: 224

Kigigha, L.T., Samson, G.A., Izah, S.C.. & Aseibai, E.R. (2018). "Microbial Assessment of Zobo Drink Sold in Some Locations in Yenagoa Metropolis, Nigeria". *EC Nutrition*, 13(7), 470 - 476.

Koketso, Z. N., Kebaneilwe, L. (2018). Microbial and chemical diversity of traditional non-cereal based alcoholic beverages of Sub-Saharan Africa, Beverages, *Science World Journal*, 4(1),36.

Musa, A.A., & Hamza, A. (2013). Comparative analysis of local prepared 'Kununaya' consumed by students of Kaduna State University, Kaduna State. *Science World Journal*, 8(2), 13-18.

Onuoha, S.C. & Fatokun, K. (2014). Effect of Citrus aurantifolia juice on the shelf-life of zobo drink produced locally in Afikpo, Ebonyi state, Nigeria. American Journal of Bioscience, 2(2),45-48.

Spencer, T. H .I., Umeh, P. O., Irokanulo, E., Baba, M. M., Spencer, B. B., Umar, A. I., Ardzard, S .A., Oderinde, S. & Onoja O. (2014). Bacterial Isolates Associated With Pelvic Inflammatory Disease Among Female Patients Attending Some Hospitals in Abuja, Nigeria. *African Journal of Infectious Dis*ease, 8(1), 9-13.

Umar, G. A., Tukur, S.I., Akensire, U. A., Adamu, Z. E., Bello, O. A., Shawulu, A. H. B. & Audu, M. (2014). Microflora of kunun zaki and sobo drinks in relation to public health in Jalingo metropolis, North-Eastern Nigeria. *International Journal of Food Research*, 1, 16- 21.

NITROUS OXIDE EMISSION ON N-FERTILIZATION IN RICE GROWN UNDER PRMSU-SM CONDITION

Romar A. Alegado[1]
Ma. Shiela G. Mendoza[2]
Ronel S. De Guzman[2*]
Myla G. Santiago[2]
Student Reseacher[1], President Ramon Magsaysay State University, Zambales, Philippines, 2207
Faculty[2], President Ramon Magsaysay State University, Zambales, Philippines, 2207

ABSTRACT
The study aims to determine the estimated nitrous oxide emission on N-fertilization in rice grown under PRMSU-SM condition. It focuses on the N residue and N_2O emission.
The result of the study showed that Treatment 4 recorded the highest amount of nitrogen in crop residue return to soil and nitrous oxide emission, which indicates that the higher the nitrogen application, the higher the N residue and N_2O emission. Also, Treatment 4 recorded the lowest soil pH level, which indicates that the higher the amount of nitrogen applied to the soil resulted in soil acidity. Moreover, the recommended application rate of fertilizer can be lessened based on the calculation to reduce the N_2O emission.
Meanwhile, researchable areas, like assessing N_2O emission in the rice field and other crops, need to be addressed.
Keyword: *Nitrous oxide emission, fertilization, residue*

A TECHNICAL STUDY ON PLUG FLOW REACTOR DESIGN AND EXPERIMENTATION FOR WASTE PLASTIC PYROLYSIS TO VALUABLE MATERIALS

Ph.D Researcher: Osman Y. Yansaneh
Supervisor: Dr. Sharif H. Zein*
Department of Chemical Engineering, Faculty of Science and Engineering, University of Hull

ABSTRACT
Climate change and groundwater contamination are typical environmental problems caused by waste plastics. Pyrolysis has shown to be a reputable process for managing these wastes using reactors. In this paper, a continuous flow, otherwise called a plug flow reactor (PFR), is designed to pyrolyse waste plastics into valuable materials. A lab furnace was used to heat the proposed PFR. The reactor pyrolyses solid waste plastics into valuable materials. To the best of the researcher's knowledge, this is a novel work owing to the extensive literature review performed and the publications made in their other works. The reactor consists of three sections: 1) inlet, 2) main chamber and 3) outlet. The main chamber with length of approximately 40 cm, a reactant velocity of 20-25 m/s and a volumetric flow rate 0.5-1 m^3/s. Single-phase products of varying crystalline structures are produced at the minimum optimum conditions of 350-600 °C reaction temperature and 100-200 min residence time. These valuable products are applicable in a wide range of applications.
***Keywords*:** *waste plastics; Climate Change; Plug Flow Reactor (PFR); Reactor Design; Pyrolysis; Valuable Materials.*

OPTIMIZATION OF BIOGAS PRODUCTION THROUGH ANAEROBIC CO-DIGESTION OF ORGANIC WASTE FROM HUMAN BODY AND PINEAPPLE PEEL

Xumay Bura Hhaygwawu[1]
Achisa C. Mecha[2]
Jacqueline K. Makatiani[3]
[1]Department of Mechanical Production and Energy Engineering, Moi University, P. O. Box 3900 – 30100 Eldoret, Kenya
[2]Department of Chemical and Process Engineering, Moi University, P. O. Box 3900 – 30100 Eldoret, Kenya
[3]Depatment of Biological Science, Moi University, P. O. Box 3900 – 30100 Eldoret, Kenya

ABSTRACT
Treatment of municipal wastewater (MWW) is important to meet the required discharge standards. Onsite wastewater treatment does not need energy but produces, offsite wastewater treatment need energy and produces. This study evaluated the anaerobic mono-digestion and co-digestion of organic waste from human body (OWHB) and pineapple peel (PP) for biogas production. Urine, faeces and PP showed high total volatile solids (TVS) values of 96.56±0.5%, 82.65±0.7% and 93.56±0.6% and biodegradability index (BI) of 1.5:1, 2.1:1 and 1.6:1 respectively. Biogas production was done using batch feeding system (BFS) and continuous feeding system (CFS). The production started with BFS by mono-digestion of fresh faeces (FF), the result was 150mL for FF under an average psychrophilic temperature of 18 ± 1^0C. The mixing ratio was varied from 1:1 to 1:4, the organic loading rate (OLR) was kept at 84g TS for all set ups, Ratio 1:2.5 FF:PP produced maximum cumulative biogas of 2,379mL but production for all set ups ended in short period with decreasing order as retention time (RT) increase. The temperature was then changed to mesophilic at 35^0C, the quick decline in production was observed, on day 3 pH was observed to be 4.9. 20ml of urine was added on a daily basis and production raised to 8,321mL. pH was adjusted using 1.0MNaOH and 1.0MHCl in the range of 4.4 to 8.6 but the production ended in 4 days in decreasing order, the difference between pH after anaerobic digestion was very small. It was found out zwitterions from amino acids can adjust pH to the certain values at isoelectric point (IEP). The system of production was changed from FF to OWHB by using CFS at OLR of 18.2TSg/day, the production showed an increase for few days and become stable. It was concluded that, zwitterions are new parameters that affects biogas production and IEP for this research was 5.97±0.68. After neutralizing the zwitterionic inhibition, biogas production was optimized by 108 folds for mono-digestion of at psychrophilic temperature, 199 folds for co-digestion at psychrophilic temperature and 441 folds for co-digestion at mesophilic temperature.
Keywords: Anaerobic Co-digestion, OWHB, Fresh Faeces, Urine, Pineapple Peel, Temperature, Zwitterions, Isoeletric Point.

1. Introduction
Treatment of MWW is important to meet the required discharge standards to protect both public and environmental health [1, 2]. MWW originates from households, institutions, recreational facilities, commercial centres, and industries after it has been used for various activities [3]. About 40% of daily wastewater flow originates from toilets which consist of human faeces and urine. Human faeces and urine are called OWHB [4] and contribute to 40%

of the total pollution in wastewater treatment plants (WWTPs) [5, 6], and they are carriers of disease causing organisms [7].

There are two types of WWTPs, onsite wastewater treatment plants (OnWWTPs), where treatment plants (TPs) are located at the site of production in small plants like toilets, septic tanks and waste stabilization ponds. Onsite wastewater consist of faeces, urine, and water therefore can be treated biologically [8, 9]. Offsite wastewater treatment plants (OfWWTPs) where wastewater (WW) is transported through the sewer system to large TPs far away from the site of production like waste stabilization ponds, lagoons, and upflow anaerobic sludge blanket (UASB) [10]. WW for OfWWTPs originates from households, institutions, commercial centres, recreational facilities, and industries like beverages, winery, milk, juice, e.t.c and contains hazardous contaminants that harm micro-organism and therefore affect biological treatment [3, 11]. Sludge from OnWWTPs can be transported to OfWWTPs or sanitary landfills or disposed to the environment depending on the policy and regulations of the country [12, 13, 14] or applied on land as a source of organic fertilizers [14, 15, 16]. OnWWTPs are also called low cost sanitation and do not require energy in treatment, instead they generate energy [17], they are more economical in energy production compared to OfWWTPs [18, 19, 20]. OfWWTPs are producers of energy and consumers at the same time, they are also called high cost sanitation, the cost of operation increases with the coverage [21, 22, 23].

There are two OfWWTPs, decentralized TPs that cover a small areas whereby the treatment is done near the site of production, this type of treatment has low capital and operating costs [24, 25]. Centralized TPs that cover a large areas whereby the treatment is done far away from the site of production, this type of treatment has high capital and operating costs [24 26, 27].

The sludge from OnWWTPs is called faecal sludge (FS) [28, 29], the sludge that has been conveyed through a sewer system to OfWWTPs is called sewage sludge (SS). Sewage sludge can be divided into primary sludge and secondary sludge [3]. Fresh solid from human being is called faeces [30], the mixture of faeces and urine is called OWHB [4].

The amount of OWHB produced varies from person to person depending on the eating habit, people who eat unprocessed food or food with high fibre content produces a large amount of faeces compared to people who eat processed food or food with less fibre content [31]. On average a human beings can produce 80grams of faeces per day [32] and 1.6litres of urine per day [33, 34]. The current global annual WW production was estimated to be $3.8 \times 10^{11} M^3$ [35, 36].

Due to the nature of containing pollutants and carrying pathogenic organisms, sludge treatment before disposal or reuse is mandatory to protect the public and environment health [5, 6, 7, 11]. Treatments in OfWWTPs requires energy to meet the required standards [11], high quality effluents, stringent effluent requirement, and volume of wastewater increases the energy consumption [37, 38, 39]. Energy consumption also differs depending on treatment, technology, location, and level of development [40, 41]. Aerobic treatment consumes more energy for aeration while anaerobic consumes less [42]. Energy is consumed in colder and temperate regions for heating and mixing, while in equatorial and tropical regions heating and mixing is done by free sun heat and wind flow [43]. Anaerobic digestion of OWHB is economical because it can treat WW by changing pollutants into biogas, and kill pathogenic bacteria at the same time [4, 44].

Anaerobic co-digestion consists of two or more substrates to improve the production of biogas by supplementing the nutrients that are lacking in a single substrate [45], reducing the effects of inhibitory substances and increase biogas production [45, 46, 47]. Anaerobic co – digestions of OWHB recover energy in form of biogas, remove disease causing organisms, protect public health, environmental health and receiving water bodies from pollution [48, 49].

Several studies have been conducted on co-digestion of human excreta in the past, that include: simulated human faeces [18], fresh faeces with food waste, vegetable waste, and fruits waste [19], human excreta, cow dung and wheat straw [50], human excreta and food waste [51], human faeces and poultry litter and cow dung [20]. However there was no study done on co-digestion of OWHB and pineapple peel (PP). This study explored anaerobic co-digestion of pineapple peel (PP) with OWHB to produce biogas. It aimed to produce energy directly from WWTPs for other economic activities rather than using it to treat wastewater.

2. Materials and Methods

2.1 Materials and Equipment

Materials used were fresh faeces, urine and pineapple peel, and equipment used include oven, furnace, spectrophotometry, pH meter, and electrical conductivity meter.

2.2 Methods

Fresh faeces (FFs) were collected at the entry point to the anaerobic pond of Moi University Waste Stabilization Ponds (WSPs). Market wastes (MW) were collected at Moi University market, the wastes were selected depending on their availability throughout the year. The waste selected were banana peel (BP), pineapple peel (PP) and water melon peel (WMP) and the VW selected were collard green waste (CGW), cabbage waste (CW), and spinach waste (SW).

All substrates were transported to laboratory for analysis. The following parameters were analysed moisture content (MC), total solids (TS), total volatile solids (TVS), pH, and electrical conductivity (EC) at Moi University Chemical Engineering and Chemistry laboratories. BI (Chemical oxygen demand (COD):Biochemical oxygen demand (BOD)), analysis of TS and MC was done before every set-up to determine the mixing ratio for organic loading rate to be fed into the digesters.

Samples were weighed using weight balance model 1- 800 – METTLER supplied by Mettler Toledo, TS and MC were analysed using an oven model FH-03 supplied by Mrc Laboratory Equipment, pH was analysed using pH metre model H198127 supplied by HANNA Instruments, electrical conductivity was analysed using electrical conductivity metre model CRISON CM 35 supplied by Material Laboratory Industrie, TVS was analysed using furnace model DFO-36 supplied by Digital Muffle Furnace, COD was analysed using spectrophotometry model DR-6000 supplied by HACH, and BOD was analysed using OxiTop respirometer model OxiTop-i S6 supplied by Wuxy Ianspn.

Laboratory for Analysis of Moisture Contents, Total Solids and Total Volatile Solids

Calculations of moisture contents (MC), total solids (TS), and total volatile solids (TVS) were done according to Anderson and Ingram [52] as follows:

a) Weigh an empty crucible (W_1) g.
b) Weight of crucible and sample (W_2) g.
c) Weight of crucible and samples after drying oven at 105^0C (W_3) g.
d) Weight of crucible and samples after burning in oven at 550^0C (W_4) g.

Moisture Content (MC %) $$MC = \frac{W_2 - W_3}{W_{2-W_I}} \text{ x100} \quad (1)$$

Dry Matter or TS (%) $$TS = \left(\frac{100}{100+\%MC}\right)x(W_2 - W_1) \quad (2)$$

Total Volatile Solids (TVS %) $$TVS = \frac{W_3 - W_4}{W_3 - W_1} \text{ x 100} \quad (3)$$

Batch Feeding System under Psychrophilic Temperature

Biogas was produced using different set ups, first set up was mono-digestion of separate FF and market waste (MW), mono-digestion was followed by co-digestion of FF with MW. FF was further co-digested separately with fruit wastes (FrW) and vegetable wastes (VW). VW was eliminated from the set up because of lower biogas production and FF was co-digested

separately with BP and PP, WMP were eliminated due to low C:N from the literature [53]. Co-digestion of FF with PP produced more biogas, the sizes of PP where reduced by shredding and blendered to increase the surface area for bacterial activities, the substrates were mixed well (Figure 1). The mixed FF and PP were fed into 1.5 litres water bottles (digester) with constant organic loading (OLR) of 84g dry matter (DM) or TS. Bottles were filled up with water to 1.2 litres level. The free space was left on the top of the digesters to allow room for floating substrates during anaerobic digestion, the space was also left to allow free movement of biogas from the digester to biogas holders. All digesters were set in replicates. Biogas production was measured by the liquid displacement method. All set ups were conducted under average psychrophilic room temperature of 18^0C, the digesters set ups were as follows:

a. FFs and (MW) were digested separately.
b. FFs were co-digested with VW and FrW at the ratio 1:1:1
c. FFs were co-digested with FrW at ratio of 1:1.
d. FFs were co-digested with VW at ratio of 1:1.
e. FFs were co-digested PP at ratios 1:1, 1:2, 1:3 and1:4
f. FFs were co-digested and BP at ratios 1:1, 1:2, 1:3 and1:4
g. FFs was co-digested with PP at the ratios of 1:2, 1:2.5 1:3, 1:3.5, and 1:4.

Figure 1: PPs after shredding (left) and after blendering (right)

Batch Feeding Under Mesophilic Temperature

Batch feeding system at mesophilic temperature was applied at the temperature of 35^0C at the OLR of 84g TS or dry matter (DM) under 2 scenarios, first by using urine on daily basis to neutralize the pH in digester and second by adjusting pH using 1.0M NaOH and 1.0M HCL before feeding into digester. The optimum parameter selected were pH and mixing ratios, values of pH and mixing ratios were obtained using Design Expert (Response Surface Method), the values pH were 4.4 – 8.8 and those for mixing ratios were 1:1.2 – 1:6.8. The set up consist of mono-digestion of OWHB at average psychrophilic temperature of 18^0C and co-digestion with mixing ratio of 1:2.5 (FF:PP) at psychrophilic and mesophilic temperatures of 18^0C and 35^0C respectively.

Biogas Production under Continuous Feeding System

The mode of feeding was changed from BFS to CFS to create a condition similar to that in the anaerobic pond (plug flow anaerobic pond) of the treatment plant. Volume of plug flow digester (VD) was designed by considering daily substrate input (SD) and retention time (RT) [54, 55].

$$DV = SD * RT$$
(4)

Where:
DV = Digester volume (M^3 or Litres),
SD = Daily substrate input (M^3/day or L/day),
RT = Retention time (days).

Three digesters were set, one digester with mono-digestion of OWHB at average psychrophilic temperature of 18^0C and two digesters with co-digestion of OWHB and PP at average psychrophilic temperature of 18^0C and mesophilic temperature of 35^0C. The set-up was shown in Figure 2.

The control was set for the aim of creating the condition similar to that of the real operational anaerobic pond to observe if mono-digestion of OWHB can produce biogas. Co-digestion at both psychrophilic and mesophilic temperatures were set to observe the improvement of biogas production from mono-digestion under different temperatures, the mesophilic temperature was set using water bath. The digester was fed on a daily basis and biogas production was recorded for 24hrs. 5 litres digesters were used and substrates were fed at the OLR of 18.2g/day DM, with 364ml/day of urine to keep average ratio of human urine to faeces to 1:20 [30, 31, 32, 33]. The hydraulic retention time (HRT) was set to 2 days [56, 57, 58] with maximum solid retention time (SRT) of 15 days.

Figure 2: Plug flow digester at psychrophilic temperature

3. Results and Discussion

3.1 Characterization of Substrates

It was observed that both FF and PP have high TVS of 82.65±0.39% and 93.56±0.6 respectively, but also having BI that is between the optimum ranges for degradation by microorganisms of 1.5:1 and 2:1 respectively [59, 60, 61, 62]. From these results co-digestion of FF and PP can optimize the biogas production because both of them are biodegradable and have high TVS. Results for the analysis of the parameters were shown in Table 1.

Table 1: moisture content, total solids and total volatile solids of fresh faeces and pineapple peels

Substrates	MC (%)	TS (%)	TVS (%)	pH	BI	EC (µS/m)
FFs	79.22±0.7	20.78±0.7	82.65±0.4	6.8±0.1	2.1	140.2±0.8
PPs	72.15±0.5	27.82±0.6	93.56±0.6	4.4±0.1	1.6	145.1±0.7
Urine	92.00±0.2	8.00±0.1	96.56±0.5	6.3±0.4	1.5	1280±2.0

3.2 Biogas Production Under Batch Feeding System

Biogas production for batch feeding system was done by co-digestion of FF with different substrates and ratios to find out the substrate and ratio with high production, substrates with low production were eliminated from the set-up, for all set ups the production started within 4 hours of digester set ups and ended between 6 to 16 days. Biogas production was done under average psychrophilic room temperature of 18^0C, the results for co-digestion with different substrates and ratios were shown in the Table 2.

Table 2: The results of biogas production by co-digestion with different substrates

Mono/Co-Digestion	Substrate	Mixing Ratios	Production (ML)	Retention time (Days)
Mono - Digestion	FF	-	150	6
Mono - Digestion	MW	-	0	0
Co - Digestion	FF:MW	1:1:1	1,225	14
Co - Digestion	FF:VW	1:1	357	8
Co - Digestion	FF:FrW	1:1	1,923	16
Co - Digestion	FF:BP	1:2	1,070	9
Co - Digestion	FF:PP	1:2	1,285	8

Digestate for biogas production from co-digestion of FF with MW was left in the digester for 8 months during COVID-19 outbreak but it was found to be fresh (Figure 2), this indicates that between the substrates used one has caused total inhibition of bacteria in the digester.

Figure 2: Appearance of digestate after staying in digester for 8 months

Biogas Production from Co-digestion of FF and PP at Different Ratios

The biogas production showed that; the production increased from 847mL at the ratio 1:1 to 2,379mL at the ratio 1:2.5, the production then started to decrease to 1,519mL at a ratio of 1:3 to 629mL at a ratio of 1:4. It was also observed that for all ratios, biogas production decreases

from day one to the final day of production and ends within a short period of time with different RTs, the RT was 10 days from ratio 1:1 to 1:3 but decreased to 9 and 6 days at ratios 1:3.5 to 1:4 respectively. The behaviour of production decreasing with the increase in RT is the indicator that between FF and PP there is one substance that causes the inhibition of bacteria in the digester. The cumulative amount of biogas produced and RT for each ratio has been shown in Table 3 and trend of production was shown in Figure 3.

Table 3: Amount of biogas produced and RT for each ratio

Ratio	1:1	1:1.5	1:2	1:2.5	1:3	1:3.5	1:4
Production (mL)	847	984	1,306	2,379	1,519	1,133	629
HRT (days)	10	10	10	10	10	9	6

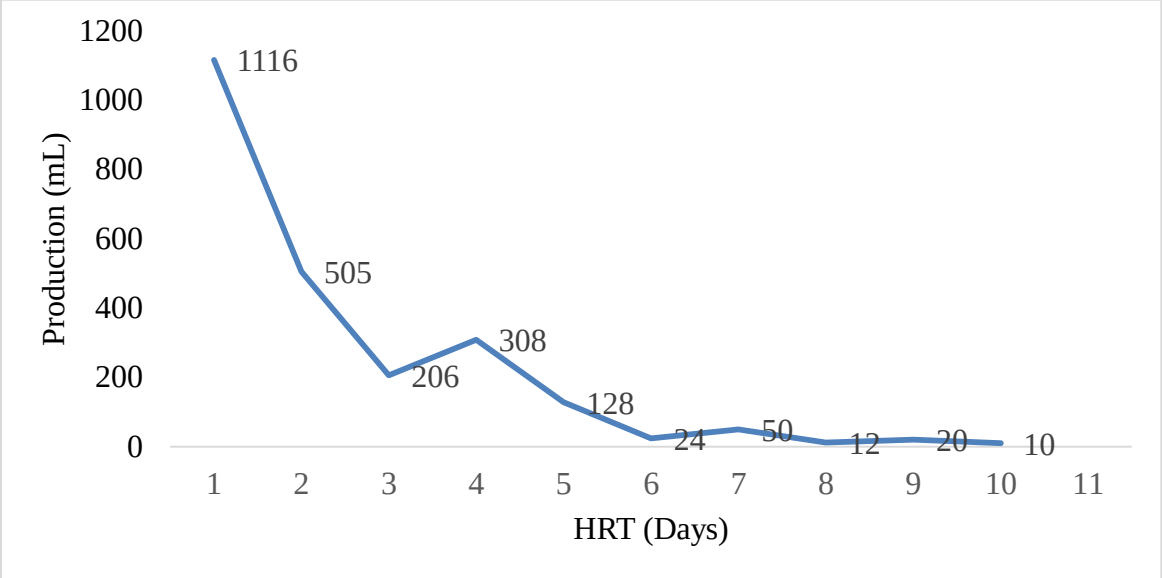


Figure 3: Biogas production from co-digestion of FFs and PP at the ratio of 1:2.5.

Biogas Production by Co-digestion of FF and PP at Ratio of 1:2.5 under Mesophilic Temperature

Under mesophilic temperature, biogas production was 193mL in day 1, it decreased to 174mL in day 2 and 94mL in day 3. pH was observed to be low at 4.9, and 20mL of urine was added to rise pH and production rise to 362mL at day 4. Without addition of urine production falls to 246mL at day 5, and 88mL at day 6. From day 7, urine was added on a daily basis until day 11 where the highest production of 1,550mL per day was observed. After day 11 the digester started to clog due to high production and it blasted 3 times, due to unclogging and blasting, the records of production were not consistent, and the production was stopped on day 15. From this set up it was clear that; the inhibitor present in the either FF or PP can be neutralized by using urine but is not suitable under BFS, it was also observed that, the time for inhibition is shorter at mesophilic temperatures compared to psychrophilic temperatures. The production has increased to 8,321mL from 2,379mL in the previous set-up. The changes in pH and biogas production were shown in the Table 4 and Figure 4

Table 4: Changes in pH in relation to addition of urine (mL)

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Urine	-	-	-	20	-	-	-	20	20	20	20	20	20	20	20
pH	-	-	4.9	5.3	5.3	5.2	5.0	5.7	5.7	6.0	6.6	6.7	6.7	6.8	6.8

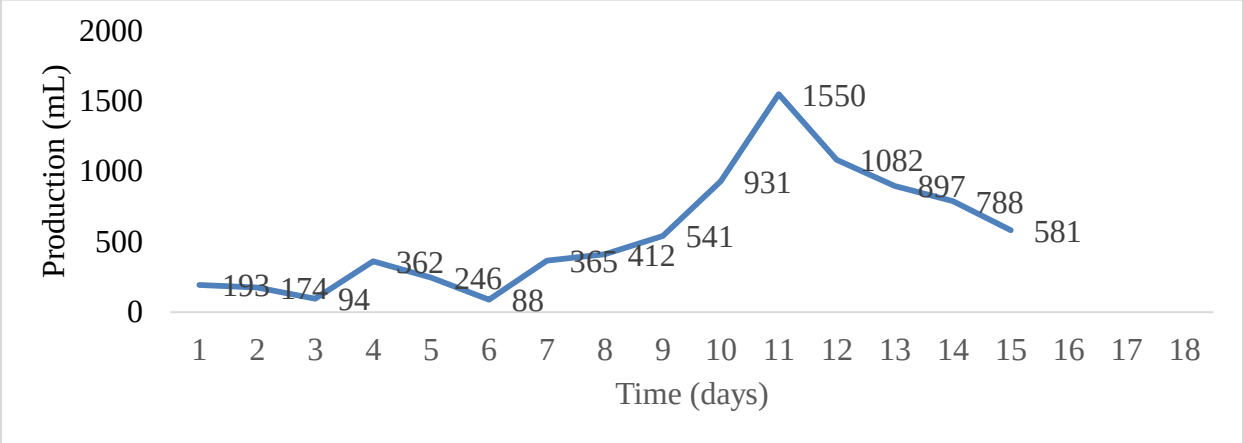


Figure 4: Biogas production from co-digestion of FFs and PP at constant mesophilic temperature of 35^0C under batch feeding system.

Biogas Production by Co-Digestion of FF with PP at Different Ratios and pH Values under Mesophilic Temperature

In this set up, the mixing ratios and pH were used as optimum conditions for biogas production, values for optimum conditions were determined by Design Expert (Surface Response Method). The values obtained were 1:1.2 – 1:6.8 for mixing ratios and 4.4 – 8.6 for pH values, pH was adjusted using 1.0MNaOH and 1.0MHCl. The digesters were set in replicate at the constant mesophilic temperature of 35^0C. Biogas production was high in the first day and decreased until the production ended in day 4. The trend of production was similar to the production from ratio 1:2.5 under mesophilic temperature. The values of pH at the end of biogas production were analysed and all values were observed to be adjusted to a narrow values with very small differences. The lowest adjusted pH value (4.4) and highest adjusted pH value (8.6) which have the difference of 4.2, have been adjusted to 5.76 and 6.07 with a small difference of 0.31 as shown in Table 5.

Table 5: Trend of biogas production with different mixing ratios and different pH values showing the changes of pH before and after anaerobic digestion

Ratios	1:2.5	1:6	1:4	1:1.2	1:6.8	1:4	1:2	1:4	1:4	1:6
Initial pH	6.83	6.57	6.84	6.93	6.85	6.82	6.89	6.87	6.88	6.91
Adjusted pH	5.0	8.0	6.5	6.5	8.0	4.4	8.0	6.5	8.6	5.0
Trend of biogas	0	1072	860	0	680	125	808	828	0	307
production in 4 days	0	190	244	0	222	0	165	247	0	220
	0	195	245	0	98	0	205	251	0	200
	0	85	180	0	92	0	110	178	155	155
pH after Digestion	5.96	6.05	6.92	5.98	5.99	5.76	5.95	5.95	6.07	5.86

From the results, it was found out that the compounds that can adjust pH with small difference are zwitterions, in the substrates used, the zwitterions are amino acids [63]. Amino acids are the end products of enteric anaerobic digestion of proteins in human intestines, amino acids

are absorbed into the blood system in the small and large intestines [64, 65, 66]. Undigested proteins and unabsorbed amino acids are transferred into the colon and leave the body as faeces [67].

During hydrolysis of amino acids to sugars, there is the point called isoelectric point (IEP) whereby amino acids can release into the aqueous solution, equal acid and base from carboxylic group (–COOH) and amine group (-NH_2) at the same time and adjust their pH to a narrow different values depending on the group of amino acid [63, 68, 69]. At IEP, the bioavailability and solubility of amino acids become very low, this decreases the biogas production because the substrates cannot be degraded biologically by microorganism in the digester [70]. From the results of this set-up it was obvious that the adjustment of pH at once cannot give optimum biogas production. To get the optimum production, pH has to be adjusted on a daily basis. This may result in high consumption of chemicals and lead to the elevation of operational costs. It was also obvious that the daily addition of urine is not suitable for BFS.

To avoid elevation of cost, the only solution was to set a plug flow digester that is equivalent to the operational plug flow anaerobic pond at laboratory scale. Urine contains urea (CH_4N_2O), under aqueous condition CH_4N_2O is hydrolysed in the presence of bacteria to form ionic ammonia nitrogen (IAN) and bicarbonates [71].

$$CH_4N_2O + 3H_2O \longrightarrow 2NH_4^+ + HCO_3^- + OH^- \quad (5)$$

The enzymes urease present in the faeces also break down urea to form free ammonia nitrogen (FAN) and carbon dioxide [72, 73, 74], FAN remains in supernatant water in the digester [69]. The equilibrium between FAN and IAN under aqueous condition can lead to the formation of hydroxyl group (-OH). The –OH in aqueous environment can help to reduce the zwitterionic impact of equal acid and base and therefore increase in more –OH will rise the pH [71, 72, 75].

$$CH_4N_2O + H_2O \longrightarrow CO_2 + 2NH_3 \quad (6)$$

$$NH_3 + H_2O \longleftrightarrow NH_4OH \quad (7)$$

$$NH_4OH \longleftrightarrow NH_4^+ + OH^- \quad (8)$$

3.3 Biogas Production under Continuous Feeding System

After changing the system of from BFS to CFS production has shown an increasing trend with time from day 1 and becomes approximately constant on day 4 for mono-digestion at psychrophilic temperature, day 5 for co-digestion at psychrophilic temperature and day 7 for co-digestion at mesophilic temperature. The set-ups were stopped at day 15 after observing that the production has remained almost constant for a substantial time. The behaviour of production to remain almost constants is an indicator that the number of bacteria present in the digester have grown enough to consume the substrates fed and convert into biogas within the same day. The cumulative biogas productions were shown in Table 6.

Table 6: Cumulative biogas productions from OWHB

Digestion System	Operating Temperature	Mean Daily production (mL)	Cumulative Production (mL)
Mono-digestion	Psychrophilic	1,153±3	16,159
Co-digestion	Psychrophilic	2,162±5	29,848
Co-Digestion	Mesophilic	4,735±29	66,177

From the study OWHB mono-digestion has optimized biogas production by 108 folds from 150mL mono-digestion of FF to 16,159mL for mono-digestion of OWHB at psychrophilic temperature, by 199 folds for co-digestion of OWHB with PP at psychrophilic temperature, and by 441 folds for co-digestion of OWHB with PP at mesophilic temperature. From the research by using faeces production of 80g/ca.day [30], OWHB operated on CFS at

psychrophilic temperature can produce 5L/ca.day for mono-digestion and 9.5L/ca.day for co-digestion with PP under psychrophilic temperature and 19L/ca.day for co-digestion with PP under mesophilic temperature. The trend of overall production for the three set-ups has been shown in the Figure 5.

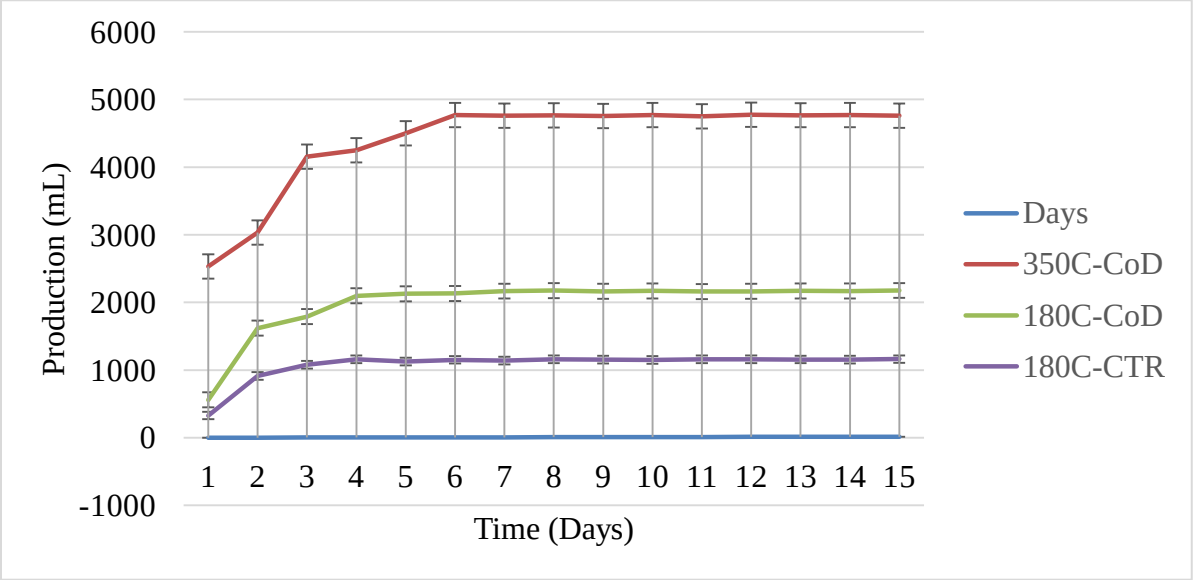


Figure 5: Trend of daily biogas production for mono-digestion at psychrophilic temperature and co-digested psychrophilic and mesophilic temperatures under CFS

The results of the research are in line with the past research by Riungu [19] whereby biogas production from anaerobic digestion of dry faeces without urine has shown a decreasing trend of biogas production with increased RT while under CFS it has shown the trend of increasing with increased RT. It is also in line with another research done by Twizerimana [76] whereby the co-digestion of human urine with cotton yarn waste increased the production of biogas from 37 days mono—digestion of cotton yarn alone to 95 days by co-digestion with human urine. Finally for biogas production from dairy industry wastewater the low pH was stabilized using NaOH [77].

4. Conclusions and Recommendations

4.1 Conclusion

From the results, it can be concluded that:

a) Zwitterion is a new inhibitory parameter that affects biogas production within a short period of time for substrates of protein origin.
b) The time for inhibition of zwitterion in anaerobic digestion of fresh faeces is shorter (4 days) at mesophilic temperatures and longer (6 -16 days) at psychrophilic temperatures.
c) For the case of this research the IEP pH of zwitterion is 5.97±0.7
d) Anaerobic digestion of OWHB for biogas production is not suitable for BFS but for CFS.
e) Mono-digestion and co-digestion of OWHB with PP at psychrophilic temperature can produce 5L/ca.day and 9.5L/ca.day respectively while co-digestion at mesophilic temperature can produce 19L/ca.day.
f) After neutralization of zwitterionic inhibition, biogas production was optimized by 108 from 150mL for mono - digestion of FF to 16,159mL for mono-digestion of OWHB under psychrophilic temperature, by 199 folds for co-digestion under psychrophilic temperature and 441 folds for co-digestion under mesophilic temperature.

g) Using OWHB for biogas production will reduce the emission of methane and other greenhouse gases into the atmosphere from wastewater treatment plants, this is in line with Agreement of Glasgow United Nations Framework Conference on Climate Change 2021 of reducing CH_4 and other greenhouse gases emissions by 30% by the year 2030 and reaching 0 emission by the year 2050 [78, 79].

4.2 Recommendation

From the findings of this research, it is recommended:

a) To do more research on the zwitterionic impacts on biogas production from other substrates of protein origin.
b) To establish zwitterion IEP for all substrates of protein origin for future application in biogas production through anaerobic digestion.

Funding

This research was funded by African Centre of Excellence in Phytochemicals, Textiles and Renewable Energy (ACE II-PTRE)

Acknowledgement

Author acknowledges the support from African Centre of Excellence in Phytochemicals, Textiles and Renewable Energy (ACE II-PTRE), Moi University, P. O. Box 3900 – 30100 Eldoret, Kenya.

References

[1] D. Ramkumara, V. Jothiprakash and B. N. Patil, "Performance assessment of sewage treatment plants using compliance index", *Journal of Water, Sanitation and Hygiene for Development,* vol. 12, no. 6, pp. 485 – 497, 2022.

[2] T. Schellenberg, V. Subramanian, G. Ganeshan, D. Tompkins and P. Rohini, "Wastewater Discharge Standards in the Evolving Context of Urban Sustainability–The Case of India", *Frontiers in Environmental Science*, vol. 8, no. 30, 2020.

[3] A. Demirbas, G. Edris and W. M. Alalayah, "Sludge production from municipal wastewater treatment in sewage treatment plant", *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, 2017.

[4] A. Regattieri, M. Bortolini, E. Ferrari, M. Gamberi and F. Piana, "Biogas Micro-Production from Human Organic Waste—A Research Proposal", *Sustainability,* vol. 10, no. 2, 2018.

[5] K. Kujawa-Roeleveld, T. Elmitwalli and G. Zeeman, "Enhanced primary treatment of concentrated black water and kitchen residues within DESAR concept using two types of anaerobic digesters", *Water Science and Technology*, vol. 53, no. 9, pp. 159 – 168, 2006.

[6] R. C. Leitão, A. C. van Haandel G. Zeeman and G. Lettinga, "The effects of operational and environmental variations on anaerobic wastewater treatment systems: A review". *Bioresource Technology*, vol. 97, pp. 1105 – 1118, 2006.

[7] S. Dumontet, A, Scopa, S. Kerje, S. and K. Krovacek, "The Importance of Pathogenic Organisms in Sewage and Sewage Sludge*", Journal of the Air & Waste Management Association,* vol. 51, no. 6, pp. 848 – 860, 2001.

[8] M. Gunady, N. Shishkina, H. Tan and C. Rodriguez, "A Review of On-Site Wastewater Treatment Systems in Western Australia from 1997 to 201", (M. K. Sridhar, Ed.), *Journal of Environmental and Public Health,* 2015.

[9] J. M. Kihila and J. G. Balengayabo, "Adaptable improved onsite wastewater treatment systems for urban settlements in developing countries", *Cogent Environmental Science*, vol. 6, 2020.

[10] A. C. Wilkie, "Anaerobic Digestion of Dairy Manure: Design and Process Considerations. In NRAES - 17, Dairy Manure Management: Treatment, Handling and Community Relations", pp. 301 – 312, Cornel University, 2005.

[11] P. Kehrein, M. van Loosdrecht, P. Osseweijer 'et al.,' "A critical review of resource recovery from municipal wastewater treatment plants – market supply potentials, technologies and bottlenecks", *Environmental Science Water Research & Technology*, vol. 6, no. 877, pp. 877 – 910. 2020.

[12] D. Capone, H. Buxton, O. Cumming 'et al.'. "Impact of an intervention to improve pit latrine emptying practices in low income urban neighborhoods of Maputo, Mozambique", *International Journal of Hygiene and Environmental Health,* vol. 226, 2001.

[13] S. Simiyu, I. Chumo and B. Mberu, "Fecal Sludge Management in Low Income Settlements: Case Study of Nakuru, Kenya", *Frontiers in Public Health,* vol. 9, 2021.

[14] WASH, "WASH in Emergencies Problem Exploration Report: Faecal Sludge Management. Humanitarian Innovation Fund", 2016.

[15] N. Andreev, M. Ronteltap, B. Boincean and P. N. Lens, "Lactic acid fermentation of human excreta for agricultural application", *Journal of Environmental Management*, vol. 206, pp. 890 – 900, 2018.

[16] K. Kinstedt, "The Application of Ecological Sanitation for Excreta Disposal in Disaster Relief: Experience, Selection and Design. Institute of Wastewater Management and Water Protection", 2012.

[17] L. Daudey, "The cost of urban sanitation solutions: a literature review", *Journal of Water, Sanitation and Hygiene for Development*, vol. 08, no. 2, pp. 176 - 195, 2018.

[18] J. Colón, A. A. Forbis-Stokes and M. A Deshusses, "Anaerobic digestion of undiluted simulant human excreta for sanitation and energy recovery in less-developed countries", *Energy for Sustainable Development*, vol. 29, pp. 57 – 64, 2015.

[19] J. Riungu, M. Ronteltap, J. B. van Lier, "Anaerobic stabilisation of urine diverting dehydrating toilet faeces (UDDT-F) in urban poor settlements: biochemical energy recovery", *Journal of Water, Sanitation and Hygiene for Development*, vol. 09, no. 2, 2019.

[20] S. Singh, N. Hariteja, S. Sharma, N. J. Raju and T. J. Prasad, "Production of biogas from human faeces mixed with the co-substrate poultry litter & cow dung", *Environmental Technology & Innovation*, vol. 3, 2021.

[21] G. R. Deshmukh, Y. G. Deshmukh and A. B. Nagdwate, "Energy Audit for Waste Water Treatment Process", *International Research Journal of Engineering and Technology*, vol. 04, no. 01, pp. 1486 – 1491, 2017.

[22] D. Panepinto, S. Fiore, M. Zappone, G. Genon and L. Meucci, "Evaluation of the energy efficiency of a large wastewater treatment plant in Italy", *Applied Energy,* vol. 161, pp. 404–41, 2016.

[23] G. Tchobanoglous H. D. Stensel, R. Tsuchihashi 'et al.' "Wastewater Engineering: Treatment and Resources Recovery", Fifth Edition, vol. 2, Metcalf & Eddy. Mc Graw Hill Education, 2014.

[24] N. N. Marleni and G. S. Raspati, "A Critical Review of Wastewater Resource Recovery Implementation in Indonesia", *Journal of Civil Engineering Forum*, vol. 6, no. 1, pp. 89 – 102. 2020.

[25] I. Obermann and K. Sattler, "Comparison of centralized, semi-centralized and decentralized sanitation systems", *First International Symposium on Urban Development: Koya as a Case Study*, pp. 159 – 167, 2013.

[26] G. Allan, I. Eromenko, M. Gilmartin, I. Kockar and P. McGregor, "The economics of distributed energy generation: A literature review", *Renewable and Sustainable Energy Reviews*, vol. 42, pp. 543–556, 2015.

[27] S. Sapmaz and I. Kilicaslan, "Biogas production from sewage sludge as a distributed energy generation element: A nationwide case study for Turkey". *Environmental*

Research & Technology, vol. 2, no. 2, 93 – 97, 2029, 2019.

[28] U. Afifah and C. R. Priadi, "Biogas Potential from Anaerobic Co-digestion of Faecal Sludge with Food Waste and Garden Waste", *Renewable Energy Technology and Innovation for Sustainable Development*, 2017.

[29] S. Septien, A. Singh, S. W. Mirara, 'et al.". "LaDePa' process for the drying and pasteurization of faecal sludge from VIP latrines using infrared radiation", *South African Journal of Chemical Engineering*, vol. 25, pp. 147-158, 2018.

[30] R. Penn, B. J. Ward, L. Strande and M. Maurer, "Review of synthetic human faeces and faecal sludge for sanitation and wastewater research", *Water Research,* vol. 132, pp. 222 - 240, 2018.

[31] A. C. Guyton, "Human physiology and mechanisms of disease", Philadelphia, USA: W. B. Saunders Co. 1992.

[32] P. Jenicek, J. Bartacek, J. Kutil, J. Zabranska, M. & Dohanyos, "Potentials and limits of anaerobic digestion of sewage sludge: Energy self-sufficient municipal wastewater treatment plant"? *Water Science & Technology,* vol. 66, no. 6, pp. 1277 – 1281, 2012.

[33] H. Braun, J. von Andrian-Werburg, O. Malisova 'et al.'. "Differing Water Intake and Hydration Status in Three European Countries—A Day-to-Day Analysis", *Nutrients*, vol. 11, no. 773, 2019.

[34] C. Rose, A. Parker, B. Jefferson and E. Cartmell, "The characterisation of faeces and urine; a review of the literature to inform advanced treatment technology", *Critical Reviews in Environmental Science and Technology,* vol. 45, pp. 1827 – 1879, 2015.

[35] S. C. Jhansi and S. K. Mishra, "Wastewater Treatment and Reuse: Sustainability Options. Consilience", *The Journal of Sustainable Development*, vol.1, no. 10, 2013.

[36] M. Qadir, P. Drechsel, B. J. Cisneros, Y. Kim, A. Pramanik, P. Mehta, and O. Olaniyan, "Global and regional potential of wastewater as a water, nutrient and energy source", *Natural Resource Forum*, pp. 40 – 51, 2020.

[37] A. Fenu, J. Roels, T. Wambecq 'et al.'. "Energy audit of a full scale MBR system", *Desalination,* vol. *262*, pp. 121 – 128, 2010.

[38] P. Jenicek, J. Kutil, O. Benes 'et al.'. "Energy self-sufficient sewage wastewater treatment plants: is optimized anaerobic sludge digestion the key?", *Water Science and Technology*, vol. 39, 15. 2013.

[39] K. Mizuta and M Shimada, "Benchmarking energy consumption in municipal wastewater treatment plants in Japan", *Water Science and Technology*, vol. 62, pp. 2256 – 2262, 2010.

[40] I. Bodik and M. Kubaská, "Energy and sustainability of operation of a wastewater treatment plant", *Environment Protection Engineering*, vol. 39, no. 2, 2015.

[41] S. C. Oliveira and M. von Sperling, "Elements for setting up discharge standards in developing countries based on actual wastewater treatment plant performance", *Water, Science & Technology*, vol. 58 no. 10, pp. 2001 – 2008, 2008.

[42] J. B. van Lier, "High-rate anaerobic wastewater treatment: diversifying from end-of-the-pipe treatment to resource-oriented conversion techniques", *Water Science & Technology*, vol. 57, no. 8, 1137–48, 2008.

[43] Y. He, Y. Zhu, J. Chen 'et al.', "Assessment of energy consumption of municipal wastewater treatment plants in China", Journal *of Cleaner Production*, vol. 228, pp. 399 – 404, 2019.

[44] SA, "The Sphere Handbook: Humanitarian Charter and Minimum Standards in Humanitarian Response", Fourth edition. Geneva, Switzerland, 2018.

[45] N. Bachmann, "Sustainable biogas production in municipal wastewater treatment plants," IEA Bioenergy, 2015.

[46] F. Hanum, L. C. Yuan, H. Kamahara 'et al.', "Treatment of Sewage Sludge Using

Anaerobic Digestion in Malaysia: Current State and Challenges", *Frontiers in Energy Research,* 2019.

[47] M. Krupp, J. Schubert and R. Widmann, "Feasibility study for co-digestion of sewage sludge with OFMSW on two wastewater treatment plants in Germany", *Waste Management*, vol. 25, pp. 393 – 399, 2005.

[48] I. Ashraf, F. Ahmad, A. Sharif, A. R. Altaf and H. Teng, "Heavy metals assessment in water, soil, vegetables and their associated health risks via consumption of vegetables, District Kasur, Pakistan", *SN Applied Sciences,* vol. 3, no. 552, 2021.

[49] R. J. Hogeboom, "The Water Footprint Concept and Water's Grand Environmental Challenges", *One Earth,* pp. 218 – 222, 2020

[50] Z. Song, J. Qin, G. Yang, Y. Feng and G. Ren, (2012). Effect of Human Excreta Mixture on Biogas Production. *Advanced Materials Research,* vol. 347 - 353, pp. 2570 – 2575, 2012.

[51] S. O. Dahunsi and U. S. Oranusi, "Co-digestion of Food Waste and Human Excreta for Biogas Production", *British Biotechnology Journal,* vol. 3, no. 4, pp. 485 – 499, 2013.

[52] J. M. Anderson and J.S. Ingram, "Tropical Soil Biology and Fertility, A Handbook of Methods," Second Edition. Wallingford: C.A.B. Internationals, 1993.

[53] A. M. Musa, C. F. Ishak, D. S. Karam and N. M Jaafar, "Effects of Fruits and Vegetable Wastes and Biodegradable Municipal Wastes Co-Mixed Composts on Nitrogen Dynamics in an OXISOL", *Agronomy,* vol. 10, no. 1609, 2020.

[54] F. N. Macharia, "Biogas Production for Domestic Use: A Flexible Learning Course", 2015.

[55] A. K. Singh and R. K. & Kaushal, "Design of Small Scale Anaerobic Digester for Application in Indian Village: A Review", *International Journal of Engineering and Applied Sciences (IJEAS)*, vol. 3, no. 8, 2016.

[56] APDG, "Design Guideline: Waste Stabilisation Ponds", Water Corporation 2009-2019, 2019.

[57] D. D. Mara and H. W. Pearson, "Design manual for waste stabilization ponds in Mediterranean countries", First Edition. Leeds, UK: Lagoon Technology International, 1998.

[58] P. C. Rodrigues, "A Design Approach for Facultative and Maturation Wastewater", Ph.D Thesis, 2019. Available on (https://dspace.lboro.ac.uk/).

[59] M. A. Aboulhassan, S. Souabi and A. Yaacoubi, "Pollution reduction and biodegradability index improvement of tannery effluents', *International Journal of Environmental Science and Technology*, vol, 5, issue 1, pp. 11 – 16, 2008.

[60] K. Chmielowski, E. Wąsik, A. Operacz 'et al.'. "Analysis of Sewage Susceptibility to Biodegradation on Example of Sewage Treatment Plant in Wodzislaw Slaski", *Infrastructure and Ecology of Rural Areas*, pp. 1427 – 1442, 2017.

[61] M. Lakhlifi, A. Elatmani, T. Elhammoumi 'et al.,' "Prediction of biodegradability ratios in wastewater treatment plant of Skhirat Morocco", *International Journal of Environmental & Agriculture Research,* vol. 3, no.12, 2017.

[62] B. Zhang, D. Ning, Y. Yang, 'et al.'. "Biodegradability of wastewater determines microbial assembly mechanisms in full-scale wastewater treatment plants", *Water Research*, 2019

[63] D. S. Dwyer, "Amino Acids: Chemical Properties" Wiley Encyclopedia of Chemical Biology, 2008.

[64] D. Dupont and Tomé, "Milk proteins: Digestion and absorption in the gastro intestinal tract" Academic Press, Milk Proteins, Third Edition, Milk Proteins From Expression to Food, Elsevier, 2020.

[65] N. G. MacFarlane, "Digestion and absorption", *Anaesthesia and Intensive Care*

Medicine, vol. 19, no. 3, pp. 125 – 127, 2018.

[66] N. van der Wielen, P. J. Moughan and M. Mensink, "Amino Acid Absorption in the Large Intestine of Humans and Porcine Models", *The Journal of Nutrition*, vol. 147, pp. 1493 – 1498, 2017.

[67] G. A. Have, M. P. Engelen, Y. C. Luiking and N. E. Deutz, N. E. "Absorption Kinetics of Amino Acids, Peptides, and Intact Proteins", *International Journal of Sport Nutrition and Exercise Metabolism*, vol. 17, 2007.

[68] A. Soult, "University of Kentucky CHEM 103 Chemistry for Allied Health. Kentucky", University of Kentucky, 2021.

[69] Y. Tanaka, H. Saito, Y. Tsutsumi 'et al.'. "Effect of pH on the interaction between zwitterions and titanium oxide", *Journal of Colloid and Interface Science*, vol.330, 138 – 143, 2009.

[70] Z. Yang, Q. Li and G. Yang, "Zwitterionic structures: from physicochemical properties toward computer-aided drug designs. A Review", *Future Medical Chemistry*, 2016.

[71] A. F. Bouwman, D. S. Lee, W. A. H. Asman, F. J. Dentener, K. W. Van Der Hoek and J. G. J. Olivier, "A global high-resolution emission inventory for ammonia", *Global Chemical Cycles*, vol.11, no. 4, pp. 561 - 587,1997.

[72] S. N. Behera, M. Sharma, V. P. Aneja and R. Balasubramanian, "Ammonia in the atmosphere: a review on emission sources, atmospheric chemistry and deposition on terrestrial bodies", *Environmental Science and Pollution Research*, 2013.

[73] Y. Chang, C. Deng, A. J. Dore and G. Zhuang, "Human Excreta as a Stable and Important Source of Atmospheric Ammonia in the Megacity of Shanghai", *PLOS ONE*, vol. 10, no. 12, 2015.

[74] K. Udert, T. A. Larsen and W. Gujer, "Fate of major compounds in source-separated urine", *Water Science & Technology*, vol. 54, no. (11–12), pp. 413 – 420, 2006.

[75] J. J. Kinhoun, A. Li, M. Lv, Y. Shi, B. Fan and T. Qian, "Human Excreta and Food Waste of a Typical Rural Area in China: Characteristics and Co-Fermentation". *International Journal of Environmental Research and Public Health*, vol. 19, 2022.

[76] M. Twizerimana, M. Marimi, X. Bura and E. O. Nganyi, "Biogas Production from Co-digestion of Cotton Yarn Waste and Human Urine*", Journal of Energy Research and Reviews*, vol. 6, no. 1, pp. 20 – 29, 2020.

[77] F. Omil, J. M, Garrido, B. Arrojo, and R. Méndez, "Anaerobic filter reactor performance for the treatment of complex dairy wastewater at industrial scale", *Water Research*, vol. 37, pp 4099 – 4108, 2003.

[78] IPCC, "The Physical Science Basis: Climate Change 2021, Working Group I contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change", Intergovernmental Panel on Climate Change, 2021.

[79] IPCC, "Mitigation of Climate Change: Climate Change 2022, Working Group III contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change", Intergovernmental Panel on Climate Change, 2022.

PROLIFERATION OF SYNTHETIC AGROCHEMICALS IN NIGERIA: IMPLICATION ON THE GOAL 14TH AND 15TH OF SUSTAINABLE DEVELOPMENT

Abdulkadir HALLIRU
Department of Science and Vocational Education, Umaru Musa Yaradua University, Katsina, Nigeria

ABSTRACT
The fact that food is inevitable for human survival makes farming imperative. However, the achievement of goal 14th (life below water) and 15th (life on land) of United Nations Sustainable Development Goals remain questionable with the continuous proliferation and unregulated utilization of artificially synthesized agrochemicals in Nigeria. Population explosion, diversification, modernization of farming, higher yield for commercial gain are some perceived forces that gear the propagation and utilization the synthetic agrochemicals in the country. Thus, statistical data shows a significant increase in the importation and use of agrochemicals with Nigeria ranked second leading importer of pesticides in Africa. Some existing and currently used synthetic agrochemicals in Nigeria and their detrimental effects on bios on land and in water were reviewed. Based on the Agenda 2030 of United Nations, this paper highlighted the objectives of the goal 14th and 15th of Sustainable Development and various problems that will hinder the achievement of these objectives were coined out. The paper also concludes and suggests on the possible ways forward based on the literatures reviewed among which; relevant government agencies like National Agency for Food and Drug Administration and Control (NAFDAC) and Standard Organization of Nigeria (SON) should review their criteria and standard for clearing and monitoring agrochemicals manufactured locally and those imported from outside the country. Agrochemicals Production Companies should review and determine safer synthetic chemicals that are target specific, greener and less harmful to the lives on land and below water with the aim of achieving the relevant SDGs.
Keywords: Proliferation, Synthetic Agrochemicals, Sustainable Development

Introduction
Food, water and air are part of the requirement for human survival on earth, the food we eat has been classified as carbohydrates, proteins, Fats, vitamins, mineral salt and water. Air remain air, no doubt, we have little option to it, but have several options to foods. These options posed challenge to humanity to plant the required variety of crops to satisfy their consumption and dietary needs. This brings the impression of farming and agriculture in every communities of the world. Agriculture is a formal term used to describe farming (planting) system. Basically, farming is an activity or business which involve growing of crops and rearing of livestock to provide food and other useful products. It can be scaled either subsistence or industrial. Therefore, agriculture is the science or practice of farming. According to the World Bank Group Archive, agricultural development is one of the most effective strategies to eradicate extreme poverty, increase shared prosperity and feed an estimated 9.7 billion people by 2050 (World Bank, 2022). It was estimated that, 70 percent of Nigeria's population largely depends on agriculture and its products for their survival and livelihood (Olukunle, 2013; Liverpool-Tasie, Kuku, Ajibola, 2011). It comprises of several activities which can be broadly categorized into three operations: pre-planting operations (activities before the planting any crop seeds, e.g. bush or land clearance, harrowing etc.),

planting operations (activities during the actual cropping) and post-planting operations (activities after planting when the crop ripe, e.g. harvesting).

Since the Industrial Revolution in the 18th and 19th century, there has been several innovations and transformations in the different sectors of production, agricultural operations inclusive. One of which the remarkable transformation in the use of crude farming tools to mechanized farming. Also, the use of synthetic chemicals known as agrochemicals has been recorded. In fact, like the majority of technologies used on a wide scale by humans for manufacturing, agrochemicals innovation accelerated during the industrial revolution worldwide. Copper nitrate, dilute sulfuric acid, sodium arsenate, copper sulfate and iron sulfate were common insecticides. Similarly, innovative techniques of applications using misters, sprayers, and air dispersal form airplanes were employed.

Agrochemicals

The word 'agrochemicals' or 'agrichemicals' is the shorten combination of two words; 'agriculture' and 'chemicals'. They can be defined as any chemical compounds or mixture of substances intended for preventing, destroying, repelling or mitigating the effect of any vectors during farming process or develop or accelerate the quality and productivity of crops. In other words, agrochemicals typically refer to pesticides, such as fungicides, insecticides, herbicides and nematicides. they also include, synthetic fertilizers, hormones and artificial other growth promoters. Therefore, broadly agrochemicals are categorized into fertilizers and pesticides. Fertilizers are chemical compounds used for promoting plant growth by mitigating nutrient deficiency in soil. They can be organic (natural) or inorganic (non-natural). Pesticides (pest destroyers) are in form of herbicides (to destroy weeds and herbs e.g. Gramoxone and Glyphosate), insecticides (to destroy insects or their eggs and larvae, e.g. Organochlorines, Organophosphates, Carbamates and Pyrethroids), rodenticides (to prevent spread of rodents like rat, mice, e.g. Klerat), fungicides (to destroy fungi and oomycetes, e.g. Mankocide), molluscides, nematicides (to control nematodes, e.g. Furadan), Molluscicides (to control molluscs like snails and slugs e.g. Slugit). Other include, avicides, repellents and attractants. However, most of these chemicals used in agriculture are not naturally occurring but synthetically produced. In fact, their use has significantly increased the concentration of toxic materials in food and the environment, with negative effects on living things on land and in water (Damalas & Eleftherohorinos, 2011; Erhunmwunse, Dirisu & Olomukoro, 2012).

In this context, some perceived forces (factors) that gear the propagation and utilization of the synthetic agrochemicals by Nigerian farmers included population explosion, modernization, diversification and higher yield for commercial. *Population explosion;* it is evident that, the population of Nigeria was estimated to be 124 million in 2000, making it one of the fastest-growing populations in the world. By 2012, however, the population had increased to 170 million, which illustrates how food production has only slightly increased between 2000 and 2012, growing at a slower rate of 3.8%. This number gives an indicator of the rate of population growth (Aina, Falola, Amoussou, Oni & Aribisala, 2019). In order to neutralize this inequality, different measures were taken to meet the need of the population through agricultural innovations and the use of agrochemicals in order to cope. Hence, the proliferation of synthetic agrochemicals in the country. *Diversification*; to broaden the economic tentacles by government, agriculture is one of the most important sector. Therefore, to ensure productivity in this sector, government provides necessary equipment and materials, among which agrochemicals inclusive. This contribute to the importation and proliferation of these chemicals. *Modernization*; modernization in agriculture involves switching from traditional labour-based agriculture to technology-based agriculture to boost production efficiency. Use of agrochemicals is part of the technology based agricultural practice for productivity enhancement. It also involved paradigm shift from the use of crude farming tools to the use of mechanized or smart tools in farming system. Thus, modernization is perceived

to be one of the factors that lead to the proliferation of synthetic agrochemicals in Nigeria. Furthermore, commercial farmers take advantage of the use of synthetic agrochemicals especially fertilizers to achieve higher yield for commercial gains. Thus, the primary aim of using agrochemicals in agricultural system is to enhance the crops health and yield.

Proliferations of Synthetic Agrochemicals in Nigeria

Even though, over the years, agrochemicals have been used by farmers globally and reported several positive impacts on the purpose it meant to serve, several researchers reported numerous health challenges e.g. (Asogwa & Dongo, 2009). In the last decade, there has been an indication of proliferation these synthetic chemicals produced for agriculture locally and many from foreign companies worldwide, Nigeria inclusive. Some are even uncertified or fake! This can be evident from the recent alarms raised about the proliferation of these chemicals in the Nigerian market. For instance, the minister of agriculture and rural development was reported to have raised alarm on the influx of fake and contraband pesticide products in Nigerian market, during the multi-stakeholders workshop on the proposed Bill for the Establishment of the Nigerian Pesticides Council (Egboboh, 2021). More recently, Oyelere (2022) reported an effort made by the federal government of Nigeria through the ministry of agriculture and rural development on sensitization of agro-dealers stakeholders against proliferation of fake agricultural input in the country. Between 1983-1990, it was estimated that, about 15,000 metric tons of pesticides comprising about 135 pesticide chemicals are imported annually in Nigeria (Osibanjo, 2002). In 2009, it was reported that, an estimated 125,000-130,000 metric tons of pesticides are used annually into the country (Asogwa & Dongo, 2009). Comparatively, between 2008-2017, among the top ten leading importers of pesticides in Africa, Nigeria was the second. Primarily, according to the FAOSTAT data, the first ten leading importer of African countries contributed a sum of $1,305,968,305 in global pesticide market. Thus, South Africa (3.356 million $; 25.7%), Nigeria (2.060 million $; 15.8%), Ghana (1.896 million $; 14.5%), Morocco (1.496 million $; 11.5%) , Egypt (1.239 million $; 9.6%), Algeria (0.861 million $; 6.6%) ,Cameroon (0.58 million $; 4.5%), Ethiopia (0.563 million $; 4.3%), Tanzania (0.504 million $; 3.9%) and Kenya (0.501 million $; 3.8%) (Tolera, 2020).

Aside from fertilizers, some pesticides commonly used in Nigeria agriculture according to world health record and other research findings include; Aldicarb, Paraquat, Dibromochloropropane, Folpet, Pentachloronitrobenzene, Captan, Carbaryl, Pyrethroids, Mancozeb (WHO, 1990), Aldrin and Dieldrin (Hunter, Carrella, Maxwell, Stewart, & Williams, 1971), Captafol (Adam, 1983), 2,4-D, Organotin, Dicofol, Parathion, Diquat, (WHO, 1990), Barban, Benomyl, DDT, Lindane, Zineb, Malathion, Hexachlorebenzene, 2,4,5-T, Pentachlorophenol and Ethylene dibromide (Almeida, 1978; Bainova, 1982), Hydrazine and Chlordecone, Chlorophenols, 1,3-dichloropropene, Chlorophenoxy, Mirex, Nitrofen, Hexachlorocyclohexanes, Sulfallate and Toxaphene (IARC, 1988), Dinitrophenols and Dinitrocresols (Weinbach, 1957), Fenthion (Misra, Nag, Mehra, & Ray, 1985), Methyl bromide (Chavez, Hepler & Straatsma, 1985), Phenoxyacid (Dossing, 1984), Carbofuran, Chorotoluron, Cyanazine, 1,2-dibromoethane, Fenoprop, Heptachlor and Heptachlor Epoxide, Isoproturon, Methyl-parathion, Methoxychlor, Molinate, Pyripoxyfen and Metachlor (WHO, 2006). However, these chemical mixtures and compounds were reported to be the cause of several health issues ranging from metabolic issues, dermatitis, allergic reactions, inhibitions, chlorance, mutagenic, carcinogenic, kidney and liver issues, impairments, effect on the immune system, toxicity and genetic issues among others. Consequently, unregulated proliferation and unsustainable long-term effect on are perceived to be on the lives and quality of soil (farmlands), lives and quality of water (aquatic ecosystem) and Air. Coincidentally, sustainability of lives below water and lives on land is the main target of the goal 14th ang 15th of the Sustainable Development set by the United Nations. Accordingly, this paper set to x-

rays some lacunas that are based on the review believed to hinder the achievement of the goals.

Goal 14th And 15th off Sustainable Development (SDGs)

Sustainable Development is defined as the development that satisfies the demands of the present generation without jeopardizing the ability of future generations to satisfy their own needs. Hence, society can engage with the environment through sustainable development without endangering the resource's future. Thus, the process of achieving sustainability is referred to as sustainable development. Corollary, based on the changing dimension of the world, emerging forces and changes in the world order in relation to sustainability. United Nations stand to provide a means to ascertain and ensure sustainable development through sustainable development goals. Thus, Sustainable Development goals are the blueprint to achieve a better and more sustainable future for all. They target to address the global challenges humanity face among which poverty, inequality, environmental degradation, climate changes peace and justice. Basically, there are seventeen (17) goals set to achieved by the year 2030 (Figure 1).

Figure 1: The 17 Sustainable Development Goals

SDG14 (Life below Water) targets to save and economically utilize the seas, oceans & marine assets for practical improvement through: (i) Moderating the antagonistic impact of fermented (acidified) seas through effective methodologies. (ii) Expansion in sea science financing, fundamental for saving marine assets to ensured marine biodiversity growth. (iii) Challenging crumbling of beachfront waters which has become a worldwide event caused by because of contamination and seaside eutrophication (flood of supplements in water).

SDG15 (Life on Land) targets to safeguarding biodiversity of timberland, desert, and mountain eco-systems, as a degree of a total landmass through: (i) Restoration of corrupted timberlands and land lost to drought and flood (ii) Overcoming assault of introduced species and more protection of imperiled species living on land (iii) Achieving Green Chemistry by 2030.

Implications of Synthetic Agrochemicals on the 14th & 15th SDGs

This paper identified perceived implicative challenges that would inhibit the successful achievement of the 14th & 15th SDGs by the year 2030. Therefore, analytically, item (iii) of the SDG[14] focuses on the issue of contamination of water bodies which affect biodiversity tendencies. In essence, significant amounts of chemicals used in agricultural activities in various farms washed away and find their way to the existing water bodies (lakes, ponds, seas and oceans). Consequently, aquatic lives will be at jeopardy! This will also hinder the achievement of SDGs pertinent to life under water. However, continuous indiscriminate use of agrochemicals leads to discharge of pollutants and sediments into ground water system. When this contaminated and polluted water is used as source of irrigation, it contaminated the crop and transmits its ill effects in the ecosystem (Khanna & Gupta, 2018).

Item (ii & iii) of the SDG[15] focuses to safeguarding imperiled species living on land and green chemistry on flora. In line with excess utilization of agrochemicals in the farmlands, there are two possible effects viz.; effect on Non-harmful organisms (non-pest) and effect on Soil Quality. Effect on Non-harmful organisms (non-pests) is can be noted considering the fact that, some agrochemicals (pesticide, herbicide etc.) are poisonously non-selective to non-pest (non-harmful organisms). These non-pests include the human-being contacting the chemicals and other living organisms living on land and having no harm to the crops. When these kinds of chemicals are used, the lives of innocent living organism would be at risk. This is also in line with the findings Leonila (2002) that, when pesticides are applied to destroy pests and pathogens, only 15% of the applied amount hits the actual target, with the remaining 85% being distributed in soils and air and act as pollutant. On the other hand, Soil Quality is also at disadvantage side. This is because, naturally soil has its nutrients. However, when synthetic chemicals are applied continuously and unregulated, the natural productivity of the soil perished over time. Consequently, the soil will not be able to sustain the lives of the flora naturally. Thus, this will also challenge the achievement of SDGs pertinent to live on land.

Conclusion

The goal of United Nations in achieving a sustainable planet by 2030 still carries question mark. The focus of this paper is on the 14th and 15th goals out of the 17 goals of the agenda 2030 of SDGs. The review critically emphasizes on the consequences of proliferation and unregulated utilization of agrochemicals in the Nigerian farmland as threat toward achieving the specified SDGs in the country. It is also evident that Nigeria is one of the leading importers of agrochemicals in Africa. It is also evident that some agrochemicals are fake. Almost all the synthetic agrochemicals identified are associated with at least one ill effect. However, in overall sense, humanity is always at the receiving end going by the mechanism of the identified effects. This is because, humanity beneficiary to both land and water. In fact, humans are part of the lives on land. They benefit form what is sourced on the land and water. Meanwhile, any problem associated with these two spheres (land and water) will directly or indirectly affect the wellbeing of humanity. For instance, people use water for drinking and cooking, they also use some aquatic animals like fishes as source of food. When this water gets contaminated as a result of pollutant like agrochemicals, the lives in and under water will be in danger or accumulated with those chemicals and in turn some will be consume by man as food. Similarly, organisms living on land include numerous species both pest and non-pest. Pesticides target to control or kill only pest species. However, significant amount of sprayed pesticide ends up been affecting the environments and non-target organisms living on land. This translates that the lives of those innocent non-pest species including humans could be at risk. These consequences are perceived to be challenges in realizing the 14th and 15th goals of the Agenda 2030 of United Nations.

Suggestion on the Ways Forward

This paper also suggests on the possible ways forward based on the literatures reviewed as follows;

1. Relevant government agencies like National Agency for Food and Drug Administration and Control (NAFDAC) and Standard Organization of Nigeria (SON) should review their criteria and standard for clearing and monitoring agrochemicals manufactured locally and those imported from outside the country.
2. Agrochemicals Production Companies should review and determine safer synthetic chemicals that are target specific, greener and less harmful to the lives on land and below water with the aim of achieving the relevant SDGs.
3. Farmers should be enlightened on the effect of the use of those counterfeit chemicals in their farms. They should also ascertain the quality, originality and authenticity of agrochemicals to be used in order to avoid fake products that harm the ecosystem.

References

Adams, R.M. (1983). *Occupational Skin Disease*. New York, Grune and Strattion.

Aina, I.V., Falola, A., Amoussou, P.J., Oni, M.A., & Aribisala, T. (2019). The Effect of Population Growth on the Agricultural Production in Nigeria (1961-2013). *Croatian Journal of Food Science and Technology*, 11 (2):230-236.

Almeida, W.F., Demello, D. & R-puga (1978). Influence of Nutritional Status on the Toxicology of Food Additives and Pesticides. Galli, C.L. ed., *Chemical toxicology of food*, Amsterdam, Elsevier/North Holland biomedical Press, 169-184pp.

Asogwa, E. U. & Dongo, L. N. (2009). Problems Associated with Pesticide Usage and Application in Nigerian Cocoa Production: A Review. *African Journal of Agricultural Research*, 4(8):675-683.

Bainova, A. (1982). Dermal Absorption of Pesticides: Toxicology of Pesticides, World Health Organisation Regional Office for Europe, Copenhagen, Demark. (European Cooperation on Environmental Health Aspects of the Control of Chemicals, Interim Document 9), 41-53pp.

Chavez, C.T., Hepler, R.S. & Straatsma, B.R. (1985). Methyl Bromide Optic Atrophy. *American Journal of* Ophthalmology, 99, 715-719.

Damalas C.A, Eleftherohorinos I.G. (2011). Pesticide Exposure, Safety Issues, and Risk Assessment Indicators. *Int J Environ Res Public Health*, 8(5):140-219.

Dossing, M. (1984). Non-invasion Assessment of Microsomal Enzyme Activity in Occupational Medicine: Present State of Knowledge and Future Prospective: *International Archives of Occupational and Environmental Health*, 53, 205-218.

Egboboh, C. (June 25, 2021). Nanono Raises Alarm on Influx of Fake Pesticide in Nigeria. The Whistler. https://www.google.com/amp/s/thewhistle.ng/nanono-raises-alarm-on-influx-of-fake-pesticide-in-nigeria/amp/ Retrieved on 11th September, 2022.

Erhunmwunse, N.O., Dirisu, A. & Olomukoro, J.O. (2012). Implications of Pesticide Usage in Nigeria. *Tropical Freshwater Biology*, 21 (1):15- 25.

Hunter, J., Carrella, M., Maxwell, J.D., Stewart, D.A. & Williams, R. (1971). Urinary D-Glutamic-acid Excretion as Attest for Hepatic Enzymes Induction in Man. Lancet, 1, 572-575.

International Agency for Research on Cancer, (IARC). (1988). *Overall evaluation of carcinogenicity*: An Update of IARC Monographs, Lyon IARC Evaluation of Carcinogenic Risk to Human, Supply. 7, 1-42.

Khanna, R. & Gupta, S. (2018). Agrochemicals as a Potential Cause of Ground Water Pollution: A Review. *International Journal of Chemical Studies*, 6(3): 985-990.

Leonila, M.V. (2002). *Impact of Agrochemical on Soil and Water Quality*. National Crop Protection Centre, University of the Philippines at Los Banos.

Liverpool-Tasie L., Kuku O., Ajibola O. (2011). *Review of Literature on Agricultural Productivity, Social Capital and Food Security in Nigeria*. Washington, D.C.: International Food Policy Research Institute; (Nigeria Strategy Support Program working paper; no. 21).

Misra, U.K., Nag, D., Mehra, M.K. & Ray, P.K. (1985). Some Observation on the Maolua of Pesticide Workers. *Human Toxicology*, 4, 135-145.

Olukunle O.T. (2013). Challenges and Prospects of agriculture in Nigeria: The Way Forward. *J Econ Sustain Dev*, 4(16):37-45.

Osibanjo, O. (2002). *Organochlorine in Nigeria and Africa*. In: Fieldler, H. (ed.) The Handbook of Environmental Chemistry, Vol.3.PersistentOrganicPollutants (pp. 321–354). Heidelberg, Berlin: Springer-Verlag.

Oyelere, K. (Jul 25, 2022). FG Sensitises Agro-dealers Stakeholders Against Proliferation of Fake Agricultural Input. Tribune Online. https://tribuneonlineng.com/fg-Sensitises-Agro-dealers-stakeholders-against-proliferation-of-fake-agricultural-input/. Retrieved on 11th September, 2022.

Tolera, S.T. (2022). Systematic Review on Adverse Effect of Pesticide on Top Ten Importers of African Countries. *J. Appl. Sci. Environ*. Manage, 24(9):1607-1616

Weinbach, E.C. (1957). Biochemical Basis for Toxicity of Pentachlorophenol. *Proceedings of the national academy of science* (USA), 43, 393-397.

World Bank Group Archive (2022). Agriculture and Food, Washington, D.C., United State. Aril 01, 2022 Update. Retrieved from https://www.worldbank.org/en/topic/agriculture/overview.

World Health Organisation (1990). WHO Recommended Classification of Pesticide by Hazard and Guidelines to Classification 1990-1991. Unpublished WHO Document WHO/PCS/90.1. Available on Request from: Division of Environment Health, WHO, 1211 Geneva 27, Switzerland.

World Health Organisation (2006). *WHO Gives Indoor use of DDT a Clean Bill of Health for Controlling Malaria*. Division of Environmental Health, WHO, 1211 Geneva 27, Switzerland.

EFFECT OF HYDROFLUORIC ACID CONCENTRATION ON MORPHOLOGICAL AND OPTICAL PROPERTIES OF BLACK SILICON FABRICATED BY ONE-STEP ELECTROLESS SILVER-ASSISTED WET CHEMICAL ETCHING FOR SOLAR CELLS

Auwal Abdulkadir[1]
Mohd Zamir Pakhuruddin[2]

[1]Department of Physics, Umaru Musa Yar'adua University, P. M. B. 2218 Katsina, Nigeria
[2]Photovoltaic Materials and Devices, School of Physics,
Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

ABSTRACT
In this work, optimization of one-step silver-assisted wet chemical etching method to improve broadband optical absorption in crystalline silicon (c-Si) wafer is presented. The method is based on a solution of 20 mM $AgNO_3$ and HF without addition of another oxidizing agent like H_2O_2 or HNO_3. P-type (100) mono crystalline silicon wafers with 1-10 Ωcm resistivity are used. Etching time is optimized to 20 min and effect of HF concentrations (2.5 M, 5.0 M, 7.5 M and 10.0 M HF) on nanotextures formation is investigated. Results obtained using 5.0 M HF confirms suppression of surface reflection to as low as 7.4% due to presence of nanowires with 4.5 μm length and average diameter of 100 nm on the c-Si. This presents potential $J_{sc(max)}$ of up to 38.6 mA/cm^2 or 46.8% enhancement compared to 26.3 mA/cm^2 of the reference planar c-Si. Correlation has been established between HF concentration and nanowires morphology.
Keywords: Silver-assisted wet chemical etching, black silicon, light trapping, HF concentration.

1. Introduction
Crystalline silicon (c-Si) remains the dominant material with about 90% market share in the photovoltaic (PV) industry for application as absorber material in solar cell fabrication [1]. However, its indirect bandgap nature and lower optical absorption coefficient presents surface reflection of about 40.0% over the wavelength region of interest for the operation of solar cells [2]. Currently, different types of surface nanotextures are being investigated to suppress the broadband reflection from the c-Si for an improved light absorption and short-circuit current density (J_{sc}) in the solar cells [2,3]. Fabrication of nanotextures on c-Si surface normally achieved via metal assisted chemical etching (MACE) or reactive ion etching (RIE) remain the promising approaches to reduce the surface reflection for increased light absorption [4]. These approaches produce black silicon (b-Si) wafer having dense nanotextures with dimensions below the wavelength of the incident light. Due to this, incident light on b-Si surfaces undergoes refractive index grading effect when traversing from air. This effect can reduce total surface reflection from 40.0% of the planar c-Si surface down to 5.0% [5]. The enhanced light absorption in the c-Si wafer throughout 300-1100 nm wavelength region may renders high J_{sc} and higher conversion efficiency in the solar cells [6,7].
Between MACE and RIE, MACE is most appealing and promising for fabricating b-Si absorber materials as investigated in the literature [8,9]. MACE involves etching of c-Si in a HF/H_2O_2 solution in presence of a metal catalyst (eg., such as silver (Ag), gold (Au) and copper (Cu) in form of nanoparticles (NPs)). The NPs are either deposited at the surface of the c-Si material prior to etching via random deposition in aqueous solutions or physically using thermal evaporation or sputtering followed by subsequent etching in $HF:H_2O_2$ solution (two-

step MACE) [10,11]. The NPs can also be deposited concurrently during the etching process in the aqueous etching solution (one-step MACE) [12,13]. Thus, one-step MACE is more appealing approach since the whole process can be completed in a single step. Lua et. al. reported an effective and economical room temperature one-step silver-assisted chemical etching process for the synthesis of nanopore-type b-Si which significantly decreases surface reflection of c-Si (polished mono-c-Si (100) p-type boron doped with resistivity of 1–5 Ωcm) wafer surfaces within 20 min of etching [14]. In their work, the effects of the concentration of the Ag NPs (500, 50, and 5 mM), the HF and H_2O_2 concentrations in the etchant solution ($AgNO_3$: HF : H_2O_2), the HF : H_2O_2 ratio, and the etching time on the surface morphology and the corresponding surface reflection were investigated. Recently, Costa et. al. reported silver-assisted wet chemical etching of polished mono-c-Si (100) p-type with resistivity of 1 Ωcm in a solution of HF, $AgNO_3$ (0.006 M) and H_2O_2 with etching duration of 10-15 min. In their study H_2O_2 concentration was varied between 0.6–1.2 M and HF was varied between 2.0-8.0 M to realizes an effective surface reflection of as low as 3.9% [15]. However, to further cut down the fabrication cost in the b-Si formation for solar cells, it is crucial to investigates one-step silver-assisted wet chemical etching process without involving another oxidizer such as H_2O_2 or HNO_3. To date, to our best knowledge, one-step silver-assisted wet chemical etching approach using a combination of HF and $AgNO_3$ (without addition of another oxidizing agent such as H_2O_2 or HNO_3) at different concentrations of HF to produce nanowires on c-Si (on moderately doped p-type mono c-Si wafers (100), 250 μm-thick with 1–10 Ω cm resistivity) for enhanced broadband optical absorption has not been reported.

This paper presents an investigations in to the effect of HF concentrations towards fabrication of b-Si nanowires using one-step silver-assisted wet chemical etching process in aqueous solution of HF:$AgNO_3$ (without involving another oxidizer such as H_2O_2) with optimized etching duration of 20 min. This simplifies the wet chemical requirement for the etching process (hence lower cost). HF concentration is optimized to realize b-Si absorber with optimum broadband light absorption. Surface morphological and optical properties of the b-Si are investigated. Using the optical absorption results, maximum potential short-circuit current density ($J_{sc(max)}$) is calculated to relatively estimate light coupling ability of the b-Si absorber within 300-1100 nm wavelength region.

2. Materials and Methods

2.1. Fabrication of black silicon

In this experiment, p-type 250 μm-thick mono c-Si wafers with 1–10 Ω cm resistivity are used. The wafers are cleaned using Radio Corporation of America (RCA) process to remove contaminants. For the details on this process refer to our previous publication [16]. To fabricate b-Si nanotextures at varying concentrations of HF, $AgNO_3$ with 169.87 g/mol, molar weight (2.5% w/v in water, from Sigma Aldrich) and HF 20 g/mol (49%) are used. Molar ratio solution of 20 mM $AgNO_3$ with different HF concentrations; 2.5 M, 5.0 M, 7.5 M and 10.0 M HF, is prepared in a polypropylene beaker at room temperature. Then, the c-Si wafers are dipped in these solutions for 20 min of optimized etching. After the silver-assisted wet chemical etching process, the Ag NPs are removed with concentrated HNO_3 (60%) in a sonication bath for 5 min. The b-Si wafers are then dipped in HF solution to remove the thin SiO_2 grown from the Ag NPs removal step.

2.2. Characterization of black silicon

Surface morphological properties of b-Si nanowires are characterized using Field Emission Scanning Electron Microscopy (FESEM) (model: FEI Nova NanoSEM 450). For FESEM, top view, oblique angle (30^O) and cross section of the b-Si nanowires are captured and analyzed. Surface reflection of the b-Si is measured by Cary 5000 UVVISNIR spectrophotometer over 300-1100 nm wavelength region. The spectrophotometer is equipped with an integrating sphere. To assess photon management ability of the b-Si nanowires, weighted average

reflection (WAR%) is calculated by integrating the reflection of the b-Si with AM1.5G solar spectrum over 300-1100 nm wavelength region. This is expressed in Equation 2.1 [17,18]. In this equation, R(λ) is the surface reflection of the wafers and S(λ) denotes the AM1.5G solar spectrum.

$$\boldsymbol{WAR(\lambda)} = \frac{\int_{300\ nm}^{1100\ nm} R(\lambda)S(\lambda)d\lambda}{\int_{300\ nm}^{1100\ nm} S(\lambda)d\lambda} \quad (2.1)$$

Using the hemispherical reflection results, absorption (A) is calculated via A = (100−R−T)%. For this case, since c-Si wafer is opaque material, transmission (T) is assumed to be zero. From the absorption curve, potential $J_{sc(max)}$ is calculated using Equation (2.2). In this equation, q is electron charge and S(λ) is the standard spectral photon density of sunlight for AM1.5G solar spectrum [18]. Unity charge collection (i.e. internal quantum efficiency, IQE=1, using the relationship EQE = IQE x (1-R-T), which is approximately 1-R) is assumed for all samples during the calculation. From the results, potential $J_{sc(max)}$ enhancement of the fabricated b-Si samples are calculated by normalizing their potential $J_{sc(max)}$ to that of the reference planar c-Si wafer. The calculation of the potential $J_{sc(max)}$ (and its enhancement) is important in showing the impact of the enhanced optical absorption on J_{sc} enhancement assuming solar cells are fabricated on the b-Si absorber materials.

$$J_{sc(max)} = q\int_{300\ nm}^{1100\ nm} EQE(\lambda).S(\lambda)\ d\lambda \quad (2.2)$$

3. Results and discussion

The mechanism of the b-Si nanowires formation in one-step silver-assisted wet chemical etching in a solution of HF:$AgNO_3$ without addition of another oxidizing agent involves Ag NPs deposition resulted from Ag+ ions that get reduced to Ag nuclei due to capturing electrons from the valence band of locally contacted c-Si surface [19]. This happens because the metallic nanoscopic Ag nuclei is more electro negative than c-Si which causes the Ag nuclei to grow to larger NPs. Concurrent Ag NPs assisted electroless chemical etching (removal of SiO_2 under Ag NPs) by HF take place via galvanic displacement of c-Si (via formation of SiO_2 due to local oxidation of c-Si) under the vicinity of Ag NPs on the c-Si surface [20,21]. This result in the formation of initial pits on c-Si with the Ag NPs being trapped in the pits. With longer duration and as the process continues, the Ag NPs go deeper vertically into the c-Si which with longer duration results the formation of vertically aligned standing b-Si nanowires. Complete self-assembled redox reactions taking place and formation of HNO_3 due to reaction between H^+ and $(NO_3)^-$ ions during the etching process is presented in equations 3.1-3.6 [21]. The cathodic process entails the reduction of Ag^+ ion into Ag NPs on c-Si surface while the anodic process involves the oxidation of c-Si beneath the reduced Ag NPs.

$AgNo_3 \rightarrow Ag^+ + (NO_3)^-$ (Reduction reaction, (i.e, cathodic process)) (3.1)

$Ag^+ + e^- \rightarrow Ag^o(s)$ (3.2)

$HF \rightarrow F^- + H^+$ (3.3)

$H^+ + (NO_3)^- \rightarrow HNO_3$ (3.4)

$3Si + 4\ HNO_3 \rightarrow 3\ SiO_2(s) + 4\ NO + 2H_2O$ (Oxidation reaction, (i.e, anodic process)) (3.5)

$SiO_2(s) + 6HF \rightarrow H_2SiF_6 + 2H_2O$ (3.6)

From the equations, Ag^+ ions in $AgNO_3$ get reduced to Ag nanoclusters on the c-Si surface (equation 3.1-3.2) and the $(NO_3)^-$ ions react with H^+ ion resulted from HF dissolution (in equation 3.3) to form HNO_3 (equation 3.4) which is responsible for completing the reduction reaction. This happens because Ag has high electronegativity than c-Si, due to this, it injects holes into the c-Si valence band and c-Si surface that is locally in contact with Ag NPs get oxidized (equation 3.5) and subsequently the SiO_2 is then get removed by HF leaving vertically standing b-Si nanowires on c-Si surface in $H_2SiF_6 + 2H_2O$ solution (equation 3.6) [20,21]. Thus, HF concentration always determines the rate at which SiO_2 (in equation 3.5) is removed which also determines the surface morphology of the formed nanotextures.

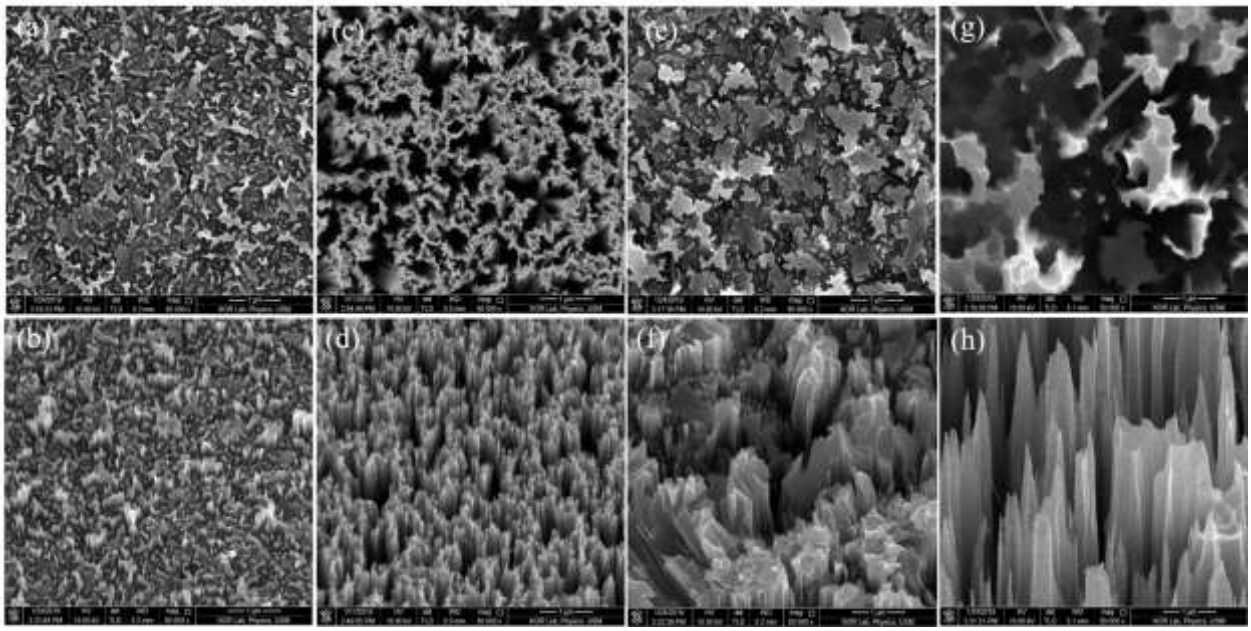


Fig. 3.1. Top and oblique (30^o) view of b-Si nanowires fabricated with different HF concentration; (a and e) 2.5 M, (b and f) 5.0 M, (c and g) 7.5 M, (d and h) 10.0 M. The scale bar is with 1 μm length.

Fig. 3.1 presents the top and oblique (30^o) view of b-Si nanowires fabricated with different HF concentrations of 2.5 M (Fig. 3.1; (a) and (b)), 5.0 M ((c) and (d)), 7.5 M ((e) and (f)) and 10.0 M ((g) and (h)). Initially, for low concentration of HF (2.5 M) during the etching process, the deposited Ag NPs with different sizes on the c-Si wafer surface cannot fabricate lots of b-Si nanotextures since the dissolution of SiO_2 under the Ag NPs cannot go faster. At the same time, as the etching of SiO_2 is not progressing, the Ag ions in the etchant solution will continuously coalesce on the previously formed Ag NPs to form larger Ag NPs (since Ag NPs on the c-Si wafer surface can transfer electrons from the c-Si to the Ag ions for Ag NPs formation). After the HF concentration is increased to 5.0 M, lengthy, dense and finely formed b-Si nanowires are visible on the c-Si wafer surface after the etching. This is resulted due to presence of available HF concentration in the etching solution which is enough to etch the formed SiO_2 faster. When HF concentration is further increased to 7.5 M the formed nanotextures appeared blurred and thicker compared to HF concentration of 5.0 M. For higher concentration of HF (10.0 M), the nanotextures are much taller with stiffer tips (needle shape) and blurred thicker bases of irregular shapes. This indicates the optimum HF concentration for formation of finely distributed b-Si nanowires has been excided [22].

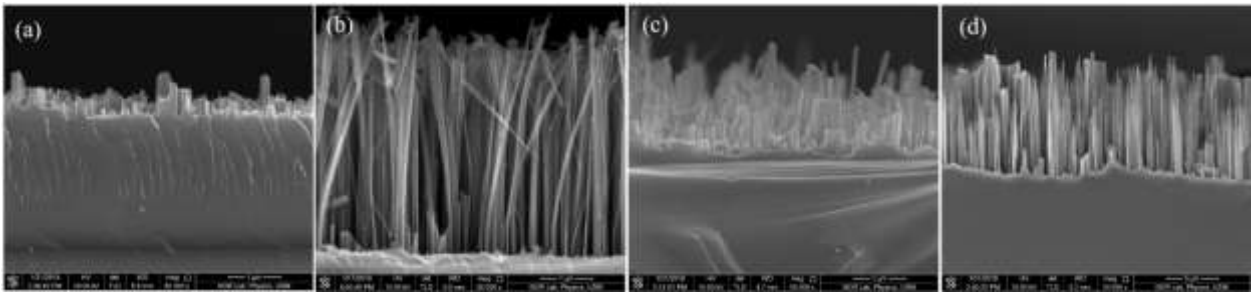
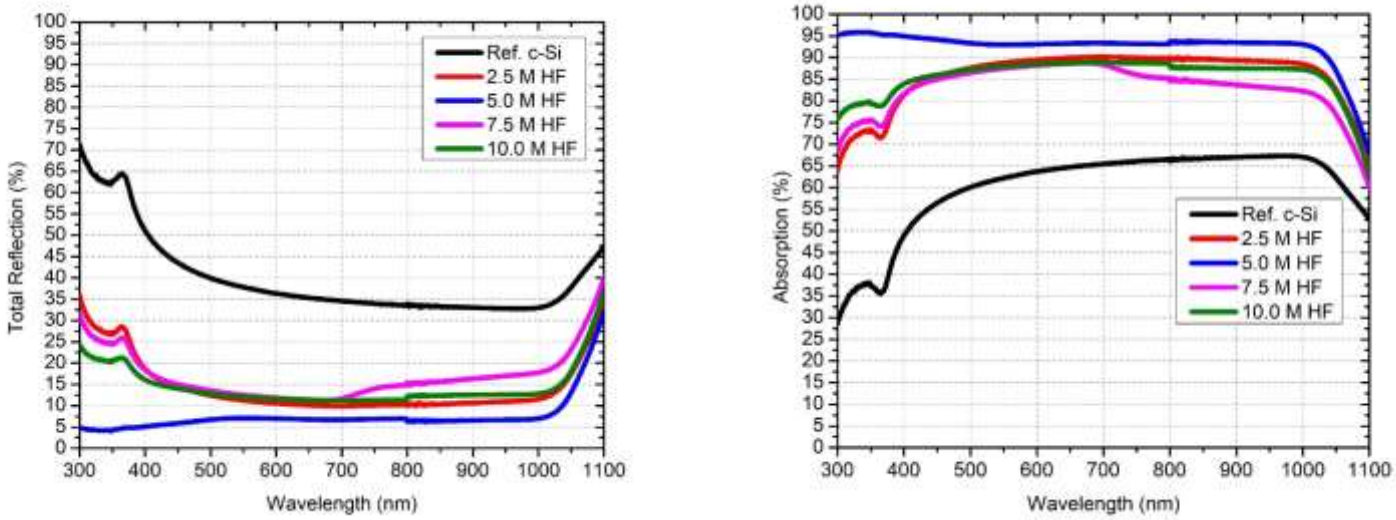


Fig. 3.2. Cross sectional SEM of b-Si nanowires fabricated with different HF concentration at 80 s; (a) 2.5 M (b) 5.0 M (c) 7.5 M (d) 10.0 M. The scale bar is with 1 μm length for (a), (b), (c) and 5 μm for (d).

Fig. 3.2 presents the cross sectional FESEM images of b-Si nanowires fabricated with different HF concentrations. From the figure, with larger Ag NPs (for 2.5 M HF), base of the b-Si nanowires has larger diameters that are nearly square in shape after the etching. This is because the diameters of the b-Si nanowires are the sinking paths of Ag NPs which are unable to go deep down into the base c-Si due to low HF concentration. Furthermore, after a certain time during the etching, the Ag NPs have sunk a bit into the c-Si wafer, such that further effective attraction of new Ag ions by Ag NPs could not be possible. Hence, many newly formed small Ag NPs deposit on some new regions at the top of c-Si wafer surface instead of on the previously formed Ag NPs (i.e at the b-Si nanowires bottoms). This causes the b-Si nanowires with varying lengths on c-Si after the etching process. This is the reason for HF concentration of 2.5 M (Fig. 4.7 (a)), b-Si nanowires with average diameter of 270 nm, varying height of 200-400 nm and thickness of 50-150 nm are seen. The reason of square like shape base diameters for this sample lies on the slower etching rate due to low HF concentration. The Ag NPs stays longer on c-Si before the SiO_2 gets etched by the HF (as highlighted earlier), due to this, already grown Ag NPs accumulates new Ag ion and becomes heavier on c-Si making their shape square like at the base contact vicinity with c-Si [22]. Another possible reason could be due to the square Si–Si bonding of a (100) c-Si wafer surface (i.e the starting substrate), making the obtained outlines of sunk Ag NPs from the chemical etching to consequently be square as well. This is because, with low HF concentration the c-Si etching rate slows down, leading the SiO_2 removal processes prefer to occur faster along the direction of Si–Si bonding because the bonding possesses a higher free energy favorable for the etching along Si–Si bonds [14]. With increasing HF concentration 5.0 M, the shape of the base average diameters (~100 nm) for b-Si nanowires with length of 4.5 μm changes slightly from square like to nearly rounded (circular). possible explanation for that, is that as the HF concentration is higher, c-Si etching rate is relatively faster, so the c-Si under the Ag NPs is easily etched with formation of circular base diameter pores which are projected outlines of the Ag NPs on the c-Si wafer surface [14]. For 7.5 M HF concentration, deterioration of b-Si nanowires starts. This indicates a begin in exceeding the maximum concentration of HF for formation of useful light trapping b-Si nanowires for solar cells. For this HF concentration b-Si nanowires with varying heights of 550 nm-900 nm, average diameter of 150 nm and thickness ranges of 50-150 nm are seen. When the HF concentration is increased to 10.0 M, not much difference is seen for the average diameter of (150 nm) compared to sample etched with HF concentration 0f 7.5 M. However, nanotexture height of 11 μm with nanotexture thickness of 50-150 nm at the base with stiffer top (needle like shapes) are seen; note image magnification of 10k was used as 50K cannot capture the FESEM images due to increased feature size for this HF concentration. This means the HF concentration is extreme and completely deteriorates the light trapping nanotextures while

still equally affecting their formation. Similar research finding has been recently reported for HF concentration exceeding 8.0 M on mono-c-Si (100) p-type with resistivity of 1 Ωcm [15]. Fig. 3.3, presents the optical reflection and absorption analysis of b-Si fabricated with different concentrations of HF compared to planar c-Si wafer. Planar reference c-Si wafer shows surface reflection of about 35% for incident light at wavelength of 600 nm with WAR of about 40.0%. For sample etched with HF concentration of 2.5 M, broadband suppression of surface reflection between 400-1000 nm wavelength region is seen even though the reflection value is a bit high (around 20.0%) at 300-400 nm wavelength region. This HF concentration presents sample with WAR value of 14.2% corresponding to an optical absorption of 90.2% at wavelength of 600 nm. This is because the b-Si nanowires begin to erupt almost uniformly despite having shorter lengths and wider square base diameters (average diameter of 270 nm, varying height of 200-400 nm).



(a) (b)

Fig. 3.3. (a) Reflectance curves for b-Si wafers etched with different HF concentrations. Reference c-Si (planar) is used for comparison. (b) Absorption curves for b-Si wafers etched with different HF concentration.

For HF concentration of 5.0 M, the WAR reduces to the minimum value of 7.4% with optical absorption of up to 93.1% at 600 nm. This is the lowest WAR realized which is attributed to the denser and taller b-Si nanowires (height of 4.5 μm) produced by the HF concentration of 5.0 M. When the HF concentration was increased to 7.5 M, surface reflection at short wavelength (300-700 nm) region increases and is even severe after wavelength region of 700 nm. The increase in surface reflection is due to the abrupt non uniform nature of the formed b-Si nanowires (refer to Fig. 3.2 (c)) for this HF concentration. This HF concentration presents sample with WAR value of 16.9% and corresponding optical absorption of 87.5% at 600 nm. For the needle shaped b-Si nanowires resulted by HF concentration of 10.0 M, WAR deviates and further drops to 14.3%. This is expected since the nanowires are nearly uniform with precisely similar shapes (steeper tips and blurred bases) all over the sample surface. Overall, with presence of b-Si nanowires, all the b-Si samples demonstrate lower broadband reflection with improved optical absorption throughout the 300-1100 nm wavelength region. The enhanced light coupling is due to the refractive index grading effect at the air-c-Si interface, owing to the presence of the nanowires [23]. When the light is incident on the c-Si surface with b-Si nanowires, the surface appears as a homogenous medium with varying refractive index (n) that changes gradually from the refractive index of air (n=1) to refractive index of

the bulk c-Si (n=3.8) This reduces broadband reflection from the c-Si surface and enhances optical absorption [23,24].

Table 1. Calculated potential $J_{sc(max)}$ and $J_{sc(max)}$ enhancement of b-Si wafers fabricated with different etching times. Potential $J_{sc(max)}$ of c-Si wafer is used as a reference.

Wafer	Potential $J_{sc(max)}$ (mA/cm^2)	Potential $J_{sc(max)}$ enhancement (%)
Reference c-Si	26.3	-
2.5 M HF	36.7	39.5
5.0 M HF	38.6	46.8
7.5 M HF	34.0	29.3
10.0 M HF	36.2	37.6

Table 1 summarizes potential $J_{sc(max)}$ and $J_{sc(max)}$ enhancement of b-Si wafers fabricated with different HF concentrations. Potential $J_{sc(max)}$ of reference c-Si wafer is 26.3 mA/cm^2. With HF concentration of 2.5 M HF, the $J_{sc(max)}$ of the b-Si wafer increases by 10.4 mA/cm^2 (compared to the reference c-Si); from 26.3 to 36.7 mA/cm^2. This represents 39.5% enhancement in the maximum achievable J_{sc}. The enhancement is attributed to the improved broadband light absorption in the b-Si when the nanowires with square bases diameters (~270 nm) and varying length of 200-400 nm are formed. After increasing the HF concentration to 5.0 M, the potential $J_{sc(max)}$ records the highest value, which is 38.6 mA/cm^2, or 46.8% enhancement. This is ~2.0 mA/cm^2 higher compared to the potential $J_{sc(max)}$ of the b-Si wafer etched with HF concentration of 2.5 M, owing to a denser formation of the nanowires with height of 4.5 μm and average base diameter of ~100 nm which resulted WAR of as low as 7.4%. The superior light coupling by the b-Si nanowires has been previously demonstrated in the absorption curves. With HF concentration of 7.5 M, the potential $J_{sc(max)}$ drops to 34.0 mA/cm^2, or 29.3% enhancement when compared with the reference planar c-Si. This is viewed as 4.0 mA/cm^2 loss in $J_{sc(max)}$ when compared with sample etched using HF concentration of 5.0 M. This is attributed to the blurred nature of the formed b-Si nanowires for this sample which causes the WAR to increase to 16.9%. For higher HF concentration that resulted needle-like b-Si nanowires on the c-Si wafer surface, calculated potential $J_{sc(max)}$ of 36.2 mA/cm^2 (37.6% enhancement compared to planar c-Si) is seen. The $J_{sc(max)}$ is still about 2.4 mA/cm^2 lower compared to $J_{sc(max)}$ of sample etched with HF concentration of 5.0 M. Thus, from this investigation HF concentration of 5.0 M is optimum for realization of useful light trapping b-Si nanowires with highest optical absorption for application in solar cells.

Despite the promising potential of improving optical absorption by the b-Si nanowires which may increase the $J_{sc(max)}$ in the c-Si absorber, it is noted that for practical application in solar cells, surface recombination of photogenerated carriers on the nanotextures is a challenge to successfully translate the enhanced broadband light absorption into improved electrical performance of the solar cells [5]. Overcoming the challenge requires an optimized deposition of surface passivation layer (such as aluminium oxide, Al_2O_3) onto the b-Si nanowires [25].

4. Conclusion

In this work, surface reflection of mono-c-Si is reduced by incorporating light trapping b-Si nanowires through electroless one-step silver assisted wet chemical etching process. The concentration of HF proved to play a key role in the etching process. It was seen that for HF concentration of 5.0 M, WAR was greatly reduced to a minimum value of 7.4%. FESEM observations reveals sample etched with HF concentration of 5.0 M is characterized with b-Si nanowires having an average length of 4.5 μm and an average diameter of ~100 nm. The

improved optical absorption resulted 46.8% enhancement in calculated potential $J_{sc(max)}$ (from 26.3 mA/cm^2 of the reference planar c-Si to 38.6 mA/cm^2). Despite the promising potential of the improved optical absorption by the b-Si nanowires, it is noted that surface recombination of photogenerated carriers on the nanotextures remains a challenge to successfully translate the enhanced broadband light absorption into improved electrical performance for practical solar cells. Overcoming the challenge requires an optimized deposition of surface passivation layer onto the b-Si nanowires.

Acknowledgement

Authors would like to acknowledge Umaru Musa Yar'adua University Katsina, TetFund Nigeria and Universiti Sains Malaysia (USM), Penang, for supporting this research.

References

[1] ITRPV, Trends & Challenges in c-Si PV - an update of the ITRPV 11 th edition (incl . maturity report), Itrpv. (2020) 1–3.

[2] X. Liu, P.R. Coxon, M. Peters, B. Hoex, J.M. Cole, D.J. Fray, Black silicon: Fabrication methods, properties and solar energy applications, Energy Environ. Sci. 7 (2014) 3223–3263. doi:10.1039/c4ee01152j.

[3] R. Liu, F. Zhang, C. Con, B. Cui, B. Sun, Lithography-free fabrication of silicon nanowire and nanohole arrays by metal-assisted chemical etching, Nanoscale Res. Lett. 8 (2013) 155. doi:10.1186/1556-276X-8-155.

[4] D. Payne, T.H. Fung, M.U. Khan, J. Cruz-Campa, K. McIntosh, M. Abbott, Understanding the optics of industrial black silicon, AIP Conf. Proc. 1999 (2018). doi:10.1063/1.5049297.

[5] J. Oh, H.C. Yuan, H.M. Branz, An 18.2%-efficient black-silicon solar cell achieved through control of carrier recombination in nanostructures, Nat. Nanotechnol. 7 (2012) 743–748. doi:10.1038/nnano.2012.166.

[6] M. Kulakci, F. Es, B. Ozdemir, H.E. Unalan, R. Turan, Application of si nanowires fabricated by metal-assisted etching to crystalline si solar cells, IEEE J. Photovoltaics. 3 (2013) 548–553. doi:10.1109/JPHOTOV.2012.2228300.

[7] H. Savin, P. Repo, G. Von Gastrow, P. Ortega, E. Calle, M. Garín, R. Alcubilla, Black silicon solar cells with interdigitated back-contacts achieve 22.1% efficiency, Nat. Nanotechnol. 10 (2015) 624–628. doi:10.1038/nnano.2015.89.

[8] C.H. Hsu, J.R. Wu, Y.T. Lu, D.J. Flood, A.R. Barron, L.C. Chen, Fabrication and characteristics of black silicon for solar cell applications: An overview, Mater. Sci. Semicond. Process. 25 (2014) 2–17. doi:10.1016/j.mssp.2014.02.005.

[9] M.F. Abdullah, M.A. Alghoul, H. Naser, N. Asim, S. Ahmadi, B. Yatim, K. Sopian, Research and development efforts on texturization to reduce the optical losses at front surface of silicon solar cell, Renew. Sustain. Energy Rev. 66 (2016) 380–398. doi:10.1016/j.rser.2016.07.065.

[10] M. Otto, M. Algasinger, H. Branz, B. Gesemann, T. Gimpel, K. Füchsel, T. Käsebier, S. Kontermann, S. Koynov, X. Li, V. Naumann, J. Oh, A. N. Sprafke, J. Ziegler, M. Zilk, and R. B. Wehrspohn, "Black silicon photovoltaics," Adv. Opt. Mater., vol. 3, no. 2, pp. 147–164, 2015.

[11] N. A. M. Noor, S. K. Mohamad, S. S. Hamil and M. Z. Pakhuruddin, "Effects of annealing temperature towards surface morphological and optical properties of black silicon fabricated by silver-assisted chemical etching," Mater. Sci. Semicond. Process., vol. 91, pp. 167–173, 2019.

[12] Y. Wang, Y.P. Liu, H.L. Liang, Z.X. Mei, X.L. Du, Broadband antireflection on the

silicon surface realized by Ag nanoparticle-patterned black silicon, Phys. Chem. Chem. Phys. 15 (2013) 2345–2350. doi:10.1039/c2cp44406b.

[13] H. Han, Z. Huang, W. Lee, Metal-assisted chemical etching of silicon and nanotechnology applications, Nano Today. 9 (2014) 271–304. doi:10.1016/j.nantod.2014.04.013.

[14] Y.T. Lu, A.R. Barron, Nanopore-type black silicon anti-reflection layers fabricated by a one-step silver-assisted chemical etching, Phys. Chem. Chem. Phys. 15 (2013) 9862–9870. doi:10.1039/c3cp51835c.

[15] I. Costa, D. Pera, J.A. Silva, Improving light capture on crystalline silicon wafers, Mater. Lett. 272 (2020) 2–5. doi:10.1016/j.matlet.2020.127825.

[16] A. Abdulkadir, A. Aziz, M.Z. Pakhuruddin, Optik Optimization of etching time for broadband absorption enhancement in black silicon fabricated by one-step electroless silver-assisted wet chemical etching, Opt. - Int. J. Light Electron Opt. 187 (2019) 74–80. doi:10.1016/j.ijleo.2019.05.019.

[17] M. Z. Pakhuruddin, J. Huang, J. Dore, and S. Varlamov, "Light absorption enhancement in laser-crystallized silicon thin films on textured glass," IEEE J. Photovoltaics, vol. 60, no. 1, pp. 159-165, 2016.

[18] A. Abdulkadir, A.A. Aziz, M.Z. Pakhuruddin, Impact of micro-texturization on hybrid micro/nano-textured surface for enhanced broadband light absorption in crystalline silicon for application in photovoltaics, Mater. Sci. Semicond. Process. 105 (2020). doi:10.1016/j.mssp.2019.104728.

[19] Z. Huang, N. Geyer, P. Werner, J. De Boor, U. Gösele, Metal-assisted chemical etching of silicon: A review, Adv. Mater. 23 (2011) 285–308. doi:10.1002/adma.201001784.

[20] T.K. Chong, K.J. Weber, K.M. Booker, A.W. Blakers, Characterization of MAE-textured nanoporous silicon for solar cells application: Optics and surface passivation, IEEE J. Photovoltaics. 4 (2014) 1235–1242. doi:10.1109/JPHOTOV.2014.2333871.

[21] M. Abouda Lachiheb, N. Nafie, M. Bouaicha, The dual role of silver during silicon etching in HF solution, Nanoscale Res. Lett. 7 (2012) 3–7. doi:10.1186/1556-276X-7-455.

[22] C. Chartier, S. Bastide, C. Lévy-Clément, Metal-assisted chemical etching of silicon in HF-H2O2, Electrochim. Acta. 53 (2008) 5509–5516. doi:10.1016/j.electacta.2008.03.009.

[23] X. He, S. Li, W. Ma, Z. Ding, J. Yu, B. Qin, J. Yang, Y. X. Zou, and J. Qiu, “A simple and low-cost chemical etching method for controllable fabrication of large-scale kinked silicon nanowires,” Mater. Lett., vol. 196, pp. 269–272, 2017.

[24] S. Li, W. Ma, X. Chen, K. Xie, Y. Li, X. He, X. Yang, and Y. Lei, “Structure and antireflection properties of SiNWs arrays form mc-Si wafer through Ag-catalyzed chemical etching,” Appl. Surf. Sci., vol. 369, pp. 232–240, 2016.

[25] K. Chen, T.P. Pasanen, V. Vähänissi, H. Savin, Effect of MACE Parameters on Electrical and Optical Properties of ALD Passivated Black Silicon, (2019). doi:10.1109/JPHOTOV.2019.2917787.

UPGRADING SCISSOR CAR JACK WITH BATTERY TO REDUCE PHYSICAL LABOR

Carlsen Norman[1]
Idham Sahaidainain[2]
Khairisyafwan Waimin[3]
Muhammad Zulkarnain Muhammad Nazlie[4]
Ernard Losumin[5]
[1,2,3,4,5] *Keningau Vocational College*

ABSTRACT

The goal of this project was to upgrade the existing scissor car jack in order to reduce the physical labor required to utilize the equipment and decrease the amount of time taken to elevate a car. The existing scissor car jacks appeared to be physically more demanding and time-consuming and majority of our target population, students from automotive technology programme at a local vocational college, complained that they could not properly access the lower parts of the car properly when using the existing car jacks. Hence, this scissor car jack was developed in which it operated through battery. The Plan-Do-Check-Action (PDCA) model was adopted in developing this project. The first stage was to identify the main problem and devise a proposed solution (Plan) and this was followed by the second stage in which the solution was further developed and tested. The third stage was the evaluation on the effectiveness of the solution (Check) and the final stage was the improvement of the project based on the findings from the previous stage. The preliminary data revealed that this project was well-received by the potential target group and if this proved to be practical, it could be used across vocational institutions.

Keywords: Scissor car jack, physical labor, automotive

EXTRACTION AND MODIFICATION OF CELLULOSE FROM *CALOTROPIS PROCERA PLANT*

Ansar Bilyaminu Adam[1]
Aliyu Danmusa Mohammed[2]
Aminu Ado Kaugama[3]
[1]Department of Chemical Science, Federal University Wukari,Taraba Nigeria
[2]Department of Chemistry, Umaru Musa Yar'adua University, Katsina State Nigeria
[3]Department of Geography and Resources Development, Federal University Wukari,Taraba Nigeria

ABSTRACT
Cellulose was extracted using 2% sodium hydroxide and 10% H_2O_2 solution from the stem of *Calotropis procera*, the stem was dried after which the lignin content was removed. Chemical modification of cellulose was successfully carried out using different amounts of sodium tripolyphosphate. Incorporation of sodium tripolyphosphate (STTP) onto the cellulose matrix, showed an increase in thickness and size of the cellulose microfibrils. The absorption band appeared at 1287 cm^{-1} in the Fourier transform infrared spectroscopy (FTIR), and is attributed to P=O stretching of the modified cellulose. Scanning electron microscopy (SEM) analysis were also used to characterize the modified cellulose.
Keywords: *Cellulose; cross linking; microfibrils; sodium tripolypohosphate; sem analysis:FTIR*

INTRODUCTION
Cellulose is the most abundant polymer on Earth, composed of glucose units, it is biodegradable and resistance to hydrolysis and hence can be regarded as a very important raw material for several purposes. Recently, cellulose has been in the public eye due to its possible use in the production of biofuels and other numerous applications [1]. Moreover, it can be chemically modified to yield cellulose derivatives. The derivatives are widely used in various industrial sectors in addition to being used as a source for commodity goods. Cellulose based materials are in the focus of numerous studies due to an increasing demand for environmental-friendly and biocompatible products in various applications. Efficient utilization of cellulose as a material source has been challenging, especially in chemical industry, it is hardly ever used as a chemical raw material because of its poor solubility resulting mainly from the highly extended hydrogen bonding of its anhydroglucose repeat units [2].
Various natural fibers such as cotton and higher plants have cellulose as their main constituent [6]. Cellulose is the main building material out of which plants are made, and plants are the primary or first link in what is known as the food chain (which describes the feeding relationships of all living things). Cellulose is the most abundant naturally occurring biopolymer [3]. It consists of long chains of anhydro-D-glucopyranose units (AGU) with each cellulose molecule having three hydroxyl groups per AGU, with the exception of the terminal ends.
Cellulose is insoluble in water and most common solvents; the poor solubility is attributed primarily to the strong intramolecular and intermolecular hydrogen bonding between the individual chains [5]. Cellulose can be chemically modified to yield derivatives which are used widely in different industrial sectors in addition to conventional applications. As an

example, in 2003, 3.2 million tons of cellulose was used as a raw material in the production of regenerated fibers and films in addition to cellulose derivatives [6].

Native cellulose has a relatively low reactivity towards adsorption or flocculation in water treatment [7], thus, the introduction of new functional groups on the surface of cellulose can increase its surface polarity and hydrophilicity, which can, in turn, enhance the adsorption of polar adsorbents and the selectivity of the cellulose for the target pollutant. However, the chemical modification of this natural polymer is slightly difficult because of the low reactivity. This is influenced also by the large number of hydrogen bonds which decrease the potential solubility in most common solvents.

Chemical modification of cellulose is performed to improve process ability and to produce cellulose derivatives (cellulosics) which can be tailored for specific industrial applications. Cellulosics are in general strong, reproducible, recyclable and biocompatible, used in various biomedical applications such as blood purification membranes and the like [8].

Cellulose derivatives are designed and fine-tuned to obtain certain desired properties. This is done by altering the inherent hydrogen bond network and by introducing different substituents. These substituents either prevented the spontaneous formation of hydrogen bonding completely or created new interactions between the cellulose chains.

Experimental

Materials

Acetic acid, nitric acid, hydrogen peroxide, sodium hydroxide and Sodium tripolyphosphate (STTP) (Sigma Aldrich) were used as received. Cellulose was extracted from the stem of *Calotropis procera*.

Samples Collection and Pre-treatment

The samples from *Calotropis Procera* were collected from Umaru Musa Yar Adua University, Katsina State, Nigeria, and identified by biology department. The samples were sun dried for 8-10 days. The leaves and small branches were separated from the stems. The stems were washed with water 4-5 times. Cleaned stems were put into an open water tank at ambient temperature for 8 days. Retting was done to loosen the bandage of fibers with stems which were expected to affect the quantity of fibers and its extraction rate. The retted stems were cleaned with water and dried thoroughly.

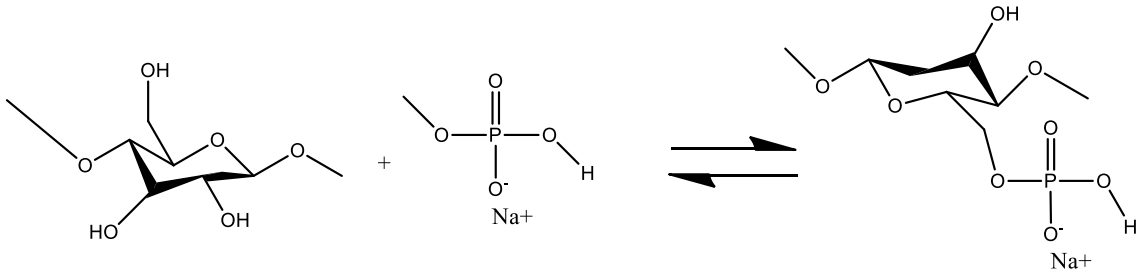
Plate. 1: Calotropis Procera Plant

Extraction of Cellulose

The extraction procedure reported by O. Brendel, 2000 [9] was used with little modification, the extraction procedure was carried out with initial 5.0 g of dried stems of *Calotropis procera*, using 2% sodium hydroxide solution and 10% H_2O_2 solution and heated at 100 ^{0}C for one hour to remove the lignin content. The extracted cellulose was washed with distilled water, centrifuged at 1000 rpm for 10 minutes and dried in an oven for 1 hour.

Phosphorylation of Cellulose

Conventional phosphorylation of starch was achieved according to the method proposed by Brendel, *et al.*, 2000 [10] with little modifications. 2.86 g of sodium tripolyphosphate (STTP) was weighed and 167 mL of distilled water added to cellulose with constant stirring. The mixture was centrifuged for 10 min at 6000 rpm in a centrifuge. The modified cellulose was dried in an oven at 40– 45 ^{0}C for 20 minutes.



FTIR

The IR absorption spectra of the sample were scanned from 4000 cm^{-1} to 600 cm^{-1} to obtain the functional groups of the cellulose and the modified sample.

Scanning Electron Microscopy

Samples extracted Substrates were directly "flat-mounted" on aluminum SEM stubs, using a two-sided adhesive film to adhere the filter to the stub, and covered by a thin gold coating [11]. Changes in morphological surface of cellulose and the modified cellulose were observed.

Results and Discussion

SEM Analysis

Scanning electron microscopes of unmodified and modified cellulose were shown in Figs1a, 1b and 1c respectively.

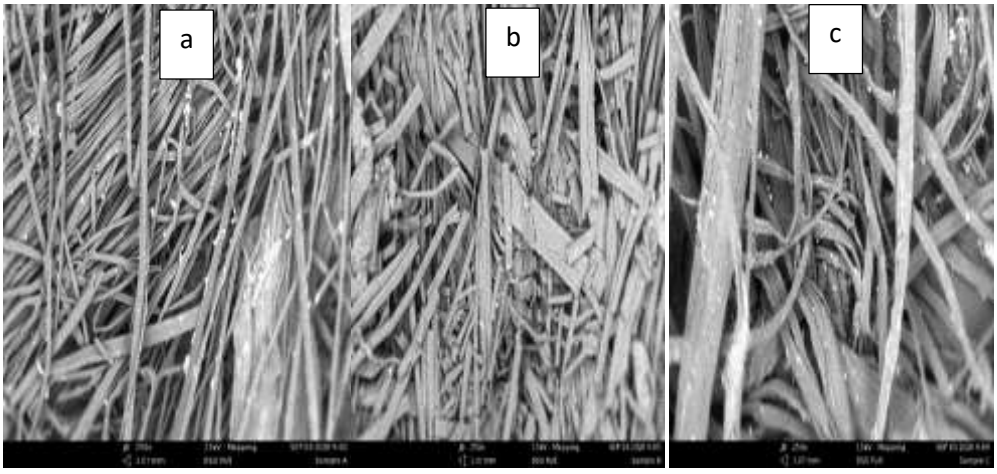


Fig. 1: SEM Images of (a) unmodified cellulose, (b) modified cellulose with 0.56g STTP and (c) modified cellulose with 2.86g STTP

The Surface morphology of the samples are studied using SEM analysis. Fig. 1 (a) shows the microfibrillar structure of cellulose network consisting of several micrometers. However, with incorporation of STTP on the cellulose matrix, there is an observed increase in thickness and

size of the cellulose microfibrils. This can be attributed to the agglomeration of STTP molecules onto the structural backbone of the cellulose molecules. Hence, Fig. 1 (c) shows thicker and larger microfibrils than Fig 1 (b), while Fig. 1 (b) shows larger microfibrils than 1 (a). Therefore, the more STTP within the cellulose structure the more the surface thickness of the microfibrils. These changes are due to more agglomeration of the particles over the surface of the cellulose backbone. Moreover, it arises from the crosslinking reactions that have taken place and, as a result, the molecular orientation of the polymer was changed. Similar observations were reported by A.D. Mohammed *et al.*, 2016 [12].

The changes observed in the morphological images confirms that structural changes have occurred due to the intermolecular bridging reactions of the cross-linking agent with sodium tripoly polyphosphate molecules.

FTIR analysis

The FTIR spectral show the vibrational frequencies of both unmodified and modified cellulose in the frequency range of 650 – 4000 cm^{-1} as shown in Figure 3 (a) and (b) respectively.

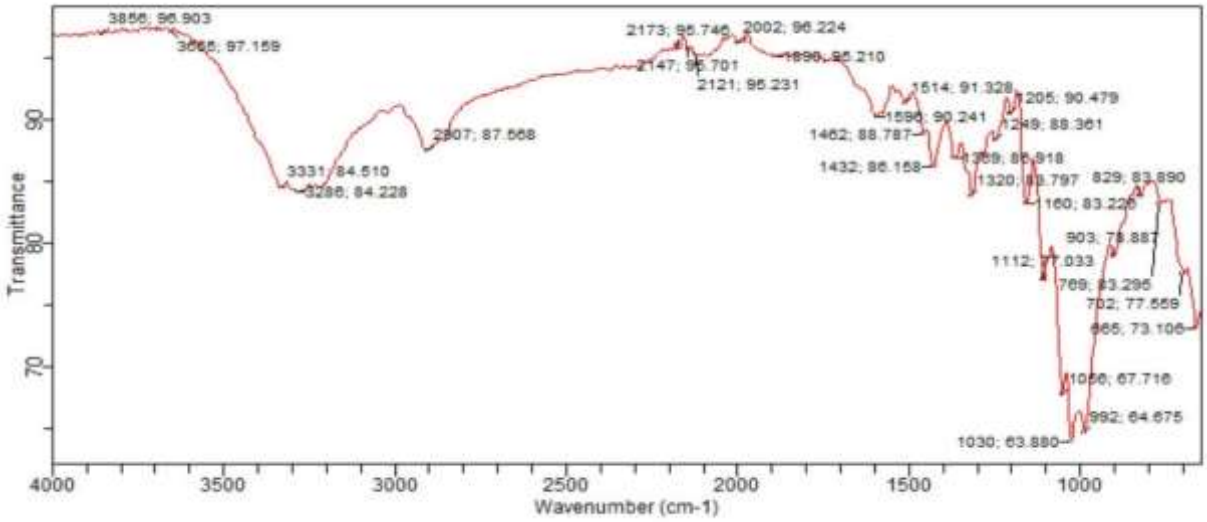


Figure. 2 (a): FTIR spectrum of unmodified cellulose

The FTIR spectra of cellulose and modified cellulose obtained from phosphorylation are shown in Fig. 2 (a) and (b) at a wave number range of 650 to 4000 cm^{-1}. The absorption peak at 3212cm^{-1} is due to -OH stretching vibration and 2807 cm^{-1} from asymmetric stretching vibration of C–H. In Fig. 2 (a), the C–O–C stretching and C–O–H bending vibrations are observed at 1030 and 1432 cm^{-1} respectively, additional absorption band in Fig. 2 (b) appeared at 12887 cm^{-1} is attributed to P=O stretching of the modified cellulose. The appearance of these bands successfully confirmed the chemical modification of *Calotropis procera* with sodium tripolyphosphate. Similar observations were reported by [13]

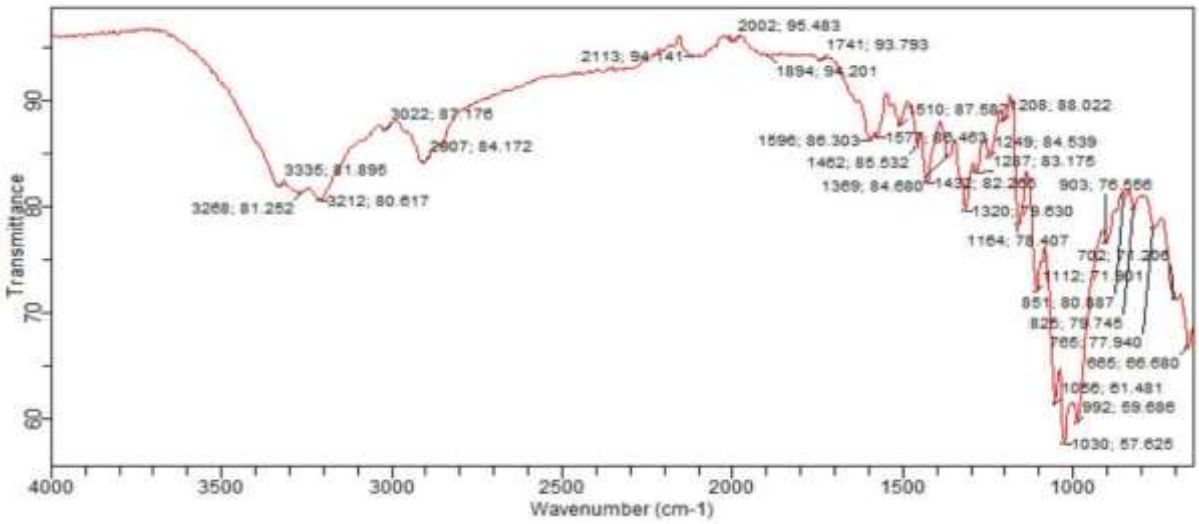


Figure. 2 (b): FTIR spectrum of modified cellulose

Conclusion

Cellulose was successfully extracted and modified using different amounts of sodium tripolyphosphate as modifying agent. The FTIR absorption band in Fig h appeared at 1287 cm^{-1} and is attributed to P=O stretching of the modified cellulose. The crosslinking reaction which resulted from the incorporation of STTP onto the cellulose matrix showed an increase in thickness and size of the cellulose microfibrils. This can be attributed to the agglomeration of STTP molecules onto the structural backbone of the cellulose molecules. This has been confirmed further from the SEM analysis.

Acknowledgements

The authors are grateful to Federal University Wukari, and Umaru Musa Yar'adua University for financial support in carrying out the research work.

References

1. Y. Habibi, "Key advances in the chemical modification of nanocelluloses," Chemical Society Reviews, vol. 43, no. 5, pp. 1519–1542, 2014.View at: Publisher Site | Google Scholar.
2. A. Elidrissi, S. El barkany , H. Amhamdi, A. Maaroufi, B. Hammouti. New approach to predict the solubility of polymers Application: Cellulose Acetate at various DS, prepared from Alfa "Stipa - tenassicima" of Eastern Morocco. J. Mater. Environ. Sci. 3 (2) (2012) 270-285. ISSN: 2028-2508.
3. B. Hinterstoisser. L. Salmen. Application of dynamic 2D FTIR to cellulose. Vibrational Spectroscopy 2000, 111–118.
4. V.V. Myasoedova. Physical chemistry of non-aqueous solutions of cellulose and its derivatives JohnWiley and Sons, Chirchester 2000.
5. A. M. Bochek: Effect of hydrogen bonding on cellulose solubility in aqueous and nonaqueous solvents. *Russian Journal of Applied Chemistry* 2003,76, 1711–1719.
6. D. Klemm. B. Heublein. H.P. Fink. A. Bohn, "cellulose: fascinating biopolymer and sustainable raw material,'' *Angewandte chemical international edition* 2005,44,22, 33583393.
7. A.W. Rudie. A. Ball. N. Patel. Ion exchange of H^+, Na^+, Mg^{2+}, Ca^{2+}, Mn^{2+},, and Ba^{2+},on wood pulp. *Journal of wood chemical technology*, 2006,26, 259.
8. I. Akira: Chemical modification of cellulose. In 'Wood and Cellulosic Chemistry' (eds.: Hon D. N-S., Shiraishi N.) Marcel Dekker, New York, 2001, 599–626.

9. O. Brendel, PPM Iannetta, D. A. Stewart: Rapid and Simple method to Isolate Pure Alpha Cellulose. *Phytochemical Analysis* 2000,11, 7-10.
10. E. F. Paschal: Phosphorylation with inorganic salt. Methods carbohydr. Chem 1964_4,296.
11. B.O Shittu. T.O.S. Popoola. O. Taiwo: Potentials of Calotropis procera Leaves for Wastewater treatment. Proceedings of the Inter. Conf. on Sci. and National Development held at University of Agriculture, Abeokuta 2004, 97.
12. A.D. Mohammed. D.A. Young. H.C.M. Vosloo: Synthesis of high-performance superabsorbent glycerol acrylate-cross-linked poly (acrylic acid). *Journal of Research Chemical Intermediates* 2016, 43, 2187-2191.
13. Y. Habibi, "Key advances in the chemical modification of nanocelluloses," Chemical Society Reviews, vol. 43, no. 5, pp. 1519–1542, 2014.View at: Publisher Site | Google Scholar.

WASTE WATER TREATMENT USING CELLULOSE CONTAINING LABILE CATIONIC GROUP

Ansar Bilyaminu Adam[1]
Aliyu Danmusa Mohammed[2]
Aminu Ado Kaugama[3]
[1]Department of Chemical Science, Federal University Wukari, Taraba Nigeria
[2]Department of Chemistry, Umaru Musa Yar'adua University, PMB 2218, Katsina, Katsina State Nigeria
[3]Department of Geography and Resources Development, Federal University Wukari, Taraba Nigeria

ABSTRACT
Cellulose containing labile cationic group was used in the adsorption studies of aqueous solution containing heavy metals. The Adsorbent Dosage showed that the amount of metal ions adsorbed decreased with increase in concentration of the initial metal ion used. At low ion concentrations, the surface active sites to the total metal ions in the solution was high and hence all metal ions interacted with the adsorbent, Cellulose containing labile Cationic group has the ability to absorb metal ions at different concentrations. The amount of metal ions adsorbed per unit mass of the adsorbent relatively decreased with increase in metal ion concentration. The results of adsorption for the following heavy metals were found to be 71.43 mg/L, 41.84 mg/L for Cd^{2+} and Cr^{2+} respectively. The adsorption equilibriums data satisfactorily fitted Langmuir isotherm equation better than Freundlich isotherm. The study indicates that the modified cellulose could be used as adsorbent for the removal of Cd^{2+} and Cr^{2+} ions from aqueous solution.
Keywords: *Adsorbent; Cellulose; Heavy metals; Labile Cationic group:*

INTRODUCTION
Adsorption is one of the chemical treatment processes commonly used for wastewater. It has a wide range of application wastewater facilities because it is efficient and simple to operate. Domestic wastewater usually contains pathogens, suspended solids, nutrients and some other organic materials [1]. The wastewater produced from different industries normally contains very fine suspended solids, dissolved solids, inorganic and organic particles, metals and other impurities. Due to very small size of the particles and presence of surface charge, the task to bring these particles closer to make heavier mass for settling and filtration becomes challenging [2].
Various traditional and advanced technologies have been utilized to remove the colloidal particles from wastewater; such as ion exchange, membrane filtration, precipitation, flotation, solvent extraction, adsorption, coagulation, flocculation and biological methods [3].
Other methods such as membrane process, adsorption, dialysis, foam flotation, osmosis, photocatalytic degradation and biological methods have been used for the removal of toxic pollutants from water and wastewater [4]. However, their applications have been restricted by many factors, such as processing efficiency, energy requirement, engineering expertise, economic benefit and infrastructure, all of which limit their use in different parts of the world.
Cationic cellulose derivatives are industrially very important compounds and have many applications in the paper industry, cosmetics and textiles. They also have potential in flotation, adsorption and in drilling fluids [5]. These substances are also utilized in the

removal of acidic dyes from aqueous effluent produced by the textile industry, thereby reducing the environmental impact of the processes [6].

Cationization of cellulose is of considerable industrial importance involving the preparation of moisturizers and conditioners for cosmetic, adsorbents and chromatography media, paper additives, pollutant adsorbents, as well as antibacterial agents in various water systems [7].

Experimental

Materials

$Cr(SO_4)_3.H_2O$, $Cd(NO_3)_2.4H_2O$, salts were purchased from Loba Chemie (England), Cellulose containing labile Cationic were synthesized in the chemistry laboratory Umaru musa Yar Adua university.

Preparation of reagents

Preparation of 1000 mg/L Cd^{2+} ions stock solution

$CdCl_2$ (2.036 g) was weighed and dissolved into 100 ml of deionized water. The solution was transferred to a 1.0 litre volumetric flack and filled to mark.

Preparation of 1000 mg/L Cr^{2+} ions stock solution

$Cr(SO_4)_3$ (2.835 g) was weighed and dissolved into 100 mL of deionized water. The solution was transferred to a 1000 mL volumetric flack and filled to mark.

Methods

Batch Equilibrium Studies

The adsorption experiments were performed by batch equilibrium method. The experiments were carried out in 250 mL conical flasks by mixing (0.58-2.88)g of the modified cellulose with 50 mL of each metal ion solutions of concentrations,($Cd^{2+}$and Cr^{2+}), 50, 175 and 300mg/L using a shaker operating at 200 rpm. The sample solutions were shaken for a time interval, and the residual metal ions in the solutions were separated by filtration and the filtrates were analyzed by using flame atomic absorption spectrophotometer (Shimdzu, 6800, Japan, 210), to determine the equilibrium metal ion concentrations. All the experiments were conducted in duplicate and averages of duplicate readings were presented. The percentage removal of metal ions and the amount of metal ions adsorbed on the modified cellulose at equilibrium (qe) were calculated using equations (4) and (5) respectively:

Percentage removal (%) = $\frac{C_o - C_e}{C_o} \times 100$ (4)

qe (mg/g) $= (c_o - c_e)\frac{V}{M}$ (5)

Where co is the initial metal ions concentration (mg/L), and ce is the equilibrium concentration of metal ions in solution (mg/L), V is the volume of metal ions solution used (L) and M is the mass of the grafted gum used (g). The equilibrium data obtained were tested using the linear forms of Langmuir and Freundlich isotherm models, as shown in equations(6) and (7), respectively, [8].

$$\frac{C_e}{q_e} = \frac{1}{q_m Q_L} + \frac{C_e}{q_m} \quad (6)$$

$$\text{Iog } q_e = \text{Iog } K_F + \frac{1}{n}\text{Iog } C_e \quad (7)$$

Results and Discussion

Adsorption Study

It was observed that the adsorption gradually increased with time and attained equilibrium after 80 minutes. The rapid intake initially could be due to the availability of the more surface sites on the adsorbent for adsorption. The plot of Ce/qe versus Ce (Figure not shown) gives straight lines with correlation coefficients of 0.99 and 0.99 for Cd^{2+} and Cr^{2+} respectively which indicate that the adsorption of the metal ions onto the adsorbent fitted the Langmuir

isotherm better than Freundlich isotherm equation. The maximum adsorption capacity of each metal ion, constants and correlation coefficients were represented in Table 2.

	Langmuir model				Freundlich model		
Metal ion	q_{max} (mg/g)	Q_L	R^2	R_L (L/mg)	K_F (mg/g)	*n*	R^2
Cd^{2+}	71.428	1.1764	0.9984	0.0010	2901.3	3.2268	0.8854
$Cr^{2}+$	41.841	0.4530	0.9973	0.0007	466.32	3.5385	0.7538

Table 1; Adsorption Study

Effect of Adsorbent Dosage

From the results obtained in figure 1 below, the amount of metal ions adsorbed decreased with increase in concentration of the initial metal ion used. At low ion concentrations surface active sites to the total metal ions in the solution was high and hence all metal ions interacted with the adsorbent and are from the solution as reported by Sweeny Mc J. D *et al.*, 2006 [9].However, at high concentrations, the driving force concentration gradient was stronger, and the amount of metal ion adsorbed per adsorbent was low. It was observed that percentage removal of Cr^{2+}, and Cd^{2+}, ions respectively, increases as the amount of adsorbents is increase. Similar result was reported by [10].

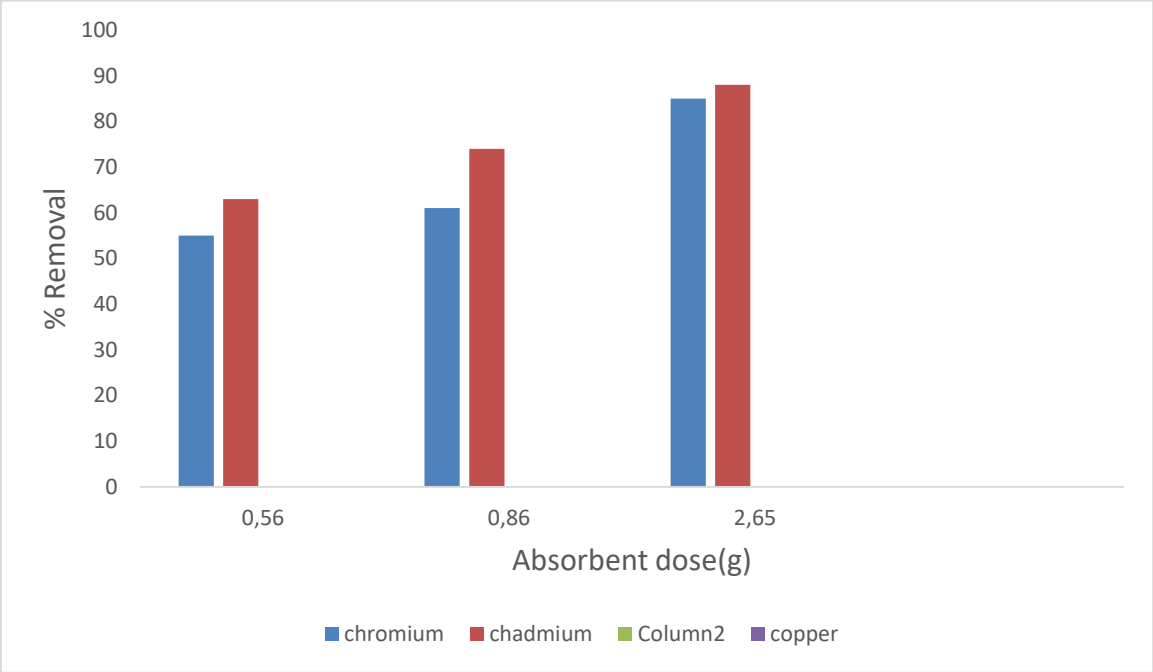


Figure 1: Effect of Adsorbent Dosage

Effect of contact time

Result in figure 2 shows the percentage removal of Cr^{2+} and Cd^{2+}, ions respectively, where amount of adsorbent 0.56 g, initial concentration (100 mg/L) by varying the contact time (10-30 min). The percentage removals of Cr^{2+} and Cd^{2+}, ions were found to be 78%, and 81% respectively. The equilibrium was achieved at 25 min. Similar result was reported [11].

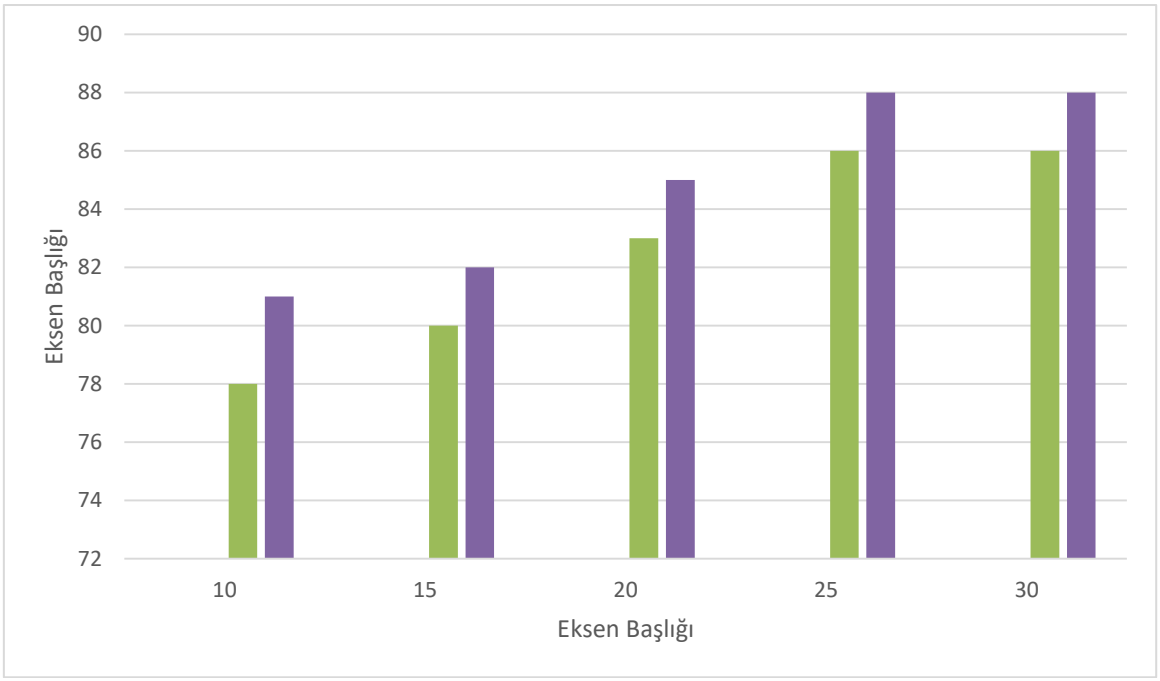


Figure 2: Effect of contact time

Effect of metal ions concentration

Figure 3 showed that the modified cellulose has the ability to absorb metal ions in different concentrations, 2.24 of modified cellulose has better absorbing capacity even in high concentration than 0.85 and 1.65g of modified cellulose. Hence the more the amount of sodium tripolyphosphate in the cellulose the higher the absorbance ability of modified cellulose. The amount of metal ions adsorbed per unit mass of the adsorbent relatively decreased with increase in metal ion concentration. At lower metal ions concentration, there was highest adsorption, and this could be due to availability of more adsorptive sites and less competition of the metal ions [12].

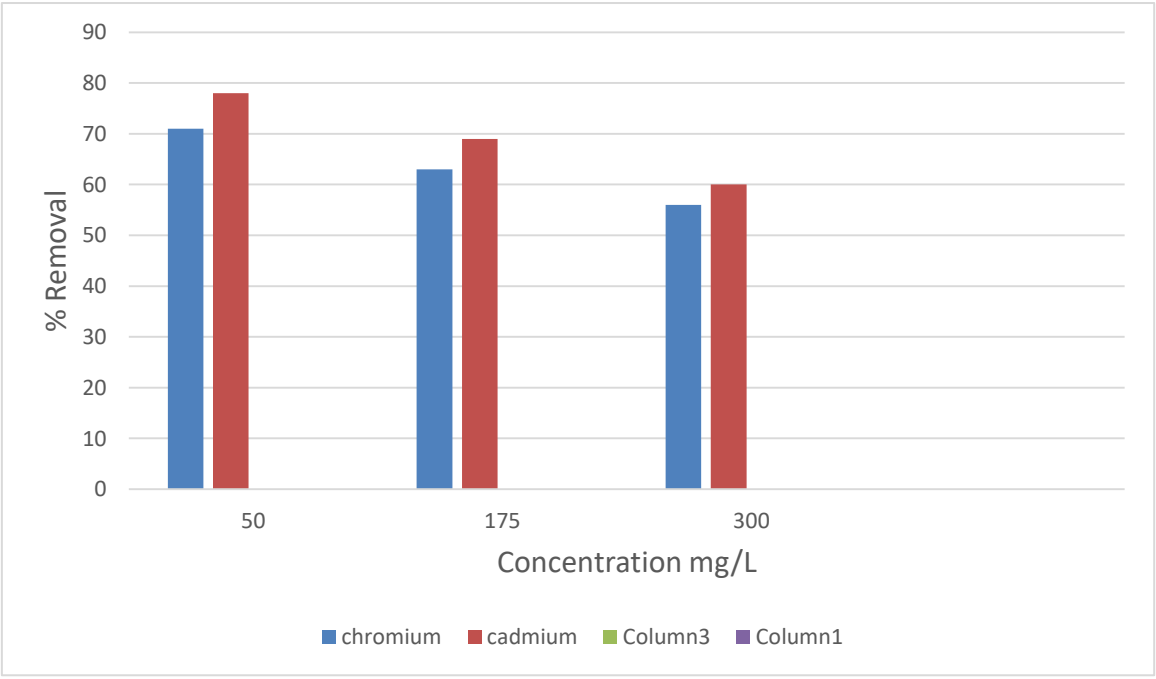


Figure 3: Effect of metal ions concentration

Conclusion

Heavy metal ions were removed from contaminated water using Cellulose containing labile Cationic group. It was found that Cellulose containing labile Cationic group has the ability to absorb metal ions at different concentrations. The amount of metal ions adsorbed per unit mass of the adsorbent relatively decreased with increase in metal ion concentration. The adsorption equilibriums data satisfactorily fitted Langmuir isotherm equation better than Freundlich isotherm. Thus, it can be concluded that the Cellulose containing labile Cationic group could be used in the treatment of toxic metals effluents polluted with Cr^{2+} and Cd^{2+} ions.

References

1. Devi, R.; Dahiya, R. COD and BOD (2008); Removal from domestic wastewater generated in decentralized sectors.Bioresour. Technol., 99, 344-349
2. Bratby, J.(2006):*Coagulation and Flocculation in Water and Wastewater Treatment*, 2nd ed. IWA Publishing, London.
3. Radoiu, M.T.;,Martin, D.I.;Calinescu, I. And Iovu, H (2004): Preparation of polyelectrolytes for wastewater treatment. Journal of Hazardous Materials.106, 27-37.
4. Pontius FW (1990): Water quality and treatment. (4^{th} edn), New York: McGrawHill, Inc. 342.
5. Abbott, A., Bell, T., Handa, S., Stoddart, B (2006): ‘’Cationic functionalization of cellulose using a chlorine based ionic liquid analogue’’ *Green chem.* 9, 784-786.
6. Blackburn, R., Burkinshaw, S. (2003): Treatment of cellulose with cationic, nucleophilic polymers to enable reactive dying at neutral pH without electrolyte addition materials. *Journal of Applied Polymer Science*, 89, 1026.
7. Hu, Z.; Soltys, (2001): Influence of particle size and pH on anaerobic degradation of cellulose by ruminal microbes. Sustainable chemistry and engineering 3 (5), 1023-1032.
8. Langmuir, I (1916): The constitution and fundamental properties of solids and liquids. *Journal American Chemical Society.* 38, 2221–2295.
9. Sweeny Mc J. D, Rowell R. M, Min SH. (2006): Effect of citric acid modification of aspen wood on sorption of copper ion. Journal of Natural Fibers. 3(1):43-58.
10. Liu, F., Luo, X., Lin, X., Liang, L., & Chen, Y. (2009): Removal of copper and lead from
aqueous solution by carboxylic acid functionalized deacetylated konjac glucomannan. *Journal of Hazardous Materials*, 171, 802–808.
11. Salisu, A., Mohd M.S., Ahmedy A.N., Ibrahim W.A.W. and Khairil J.A. (2016b): Alginate graft polyacrylonitrile beads for the Removal of lead ions from aqueous solutions, Polymer Bulletin. 73, pp. 519-537.
12. Chen, A.H., Liu, S.C. and Chen, C.Y. (2008): Comparative adsorption of Cu(II), Zn (II), and Pb(II) ions in aqueous solution on the crosslinked chitosan with epichlorohydrin. *Journal Hazardous Materials.*154, 184-191.