MICROPLASTIC POLLUTION: OCCURRENCE, BIOLOGICAL IMPACTS AND GENE EXPRESSION PROFILING OF SELECTED FISH SPECIES IN THE LAGOS LAGOON, NIGERIA

AKINHANMI, FADEKEMI OLABISI
(20PCO02218)

JUNE, 2024

MICROPLASTIC POLLUTION: OCCURRENCE, BIOLOGICAL IMPACTS AND GENE EXPRESSION PROFILING OF SELECTED FISH SPECIES IN THE LAGOS LAGOON, NIGERIA

BY

AKINHANMI, FADEKEMI OLABISI
(20PCO02218)
B.Sc. Plant Science, Olabisi Onabanjo University, Ago-Iwoye
M.Sc. Environmental Toxicology and Pollution Management, University of Lagos, Akoka

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.) IN BIOLOGY IN THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY, OTA, OGUN STATE, NIGERIA

JUNE, 2024

ACCEPTANCE

This is to attest that this thesis has been accepted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy (Ph.D.) in Biology in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria

Miss Adefunke F. Oyinloye
(Secretary, School of Postgraduate Studies) **Signature and Date**

Prof. Akan B. Williams
(Dean, School of Postgraduate Studies) **Signature and Date**

DECLARATION

I, **AKINHANMI, FADEKEMI OLABISI (20PCO02218)** declare that this research was carried out by me under the supervision of Prof. Gabriel A. Dedeke of the Department of Pure and Applied Zoology, College of Natural Sciences, Federal University of Agriculture, Abeokuta and Dr. Isaac O. Ayanda of the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria. I attest that this thesis has not been presented either wholly or partially for the award of any degree elsewhere. All the sources of data and scholarly information used in the thesis are duly acknowledged.

AKINHANMI, FADEKEMI OLABISI

Signature and Date

CERTIFICATION

We certify that this thesis titled **"MICROPLASTIC POLLUTION: OCCURRENCE, BIOLOGICAL IMPACTS AND GENE EXPRESSION PROFILING OF SELECTED FISH SPECIES IN THE LAGOS LAGOON, NIGERIA"** is an original work carried out by **AKINHANMI, FADEKEMI OLABISI (20PCO02218)**, in the Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria, under the supervision of Prof. Gabriel A. Dedeke and Dr. Opeyemi I. Ayanda. We have examined and found the work acceptable as part of the requirement for the award of Doctor of Philosophy (Ph.D.) in Biology.

Prof. Gabriel A. Dedeke
(Supervisor) **Signature and Date**

Dr. Opeyemi I. Ayanda
(Co-Supervisor) **Signature and Date**

Dr. Opeyemi I. Ayanda
(Head of Department) **Signature and Date**

Prof. Solomon O. Adewoye
(External Examiner) **Signature and Date**

Prof. Akan B. Williams
(Dean, School of Postgraduate Studies) **Signature and Date**

DEDICATION

This work is dedicated to my late mother, Mrs. Mofolorunsho Omowunmi Margret Akinhanmi.

ACKNOWLEDGMENTS

All glory to God Almighty, the giver of life and grace, for the privilege to begin, progress, and complete this doctoral program. Thank you, Lord, for making this dream a reality.

I acknowledge the Chancellor of Covenant University, Dr. David O. Oyedepo, for the inspiration and obedience to establish this royal citadel of learning.

My appreciation also goes to the Vice–Chancellor, Prof. Abiodun H. Adebayo, Deputy Vice-Chancellor, Prof. Olujide A. Adekeye, and the Registrar, Mrs. Regina A. Tobi-David, for their dedication and commitment towards training young and upcoming academics. My gratitude also goes to the Dean of Postgraduate School, Prof. Akan B. Williams; the Sub-dean of Postgraduate School, Dr. Hezekiah O. Falola; the Dean of College of Science and Technology, Prof. Timothy A. Anake and the Dean of Student Affairs, Covenant University, Mrs. Olushola E. Coker for all your efforts and readiness to guide towards a successful academic achievement.

I would like to express my profound gratitude to the Head of the Department of Biological Sciences, Dr. Opeyemi I. Ayanda, and the immediate past Head of the Department of Biological Sciences, Prof. Solomon U. Oranusi for their moral supports throughout the period of this programme. My special appreciation goes to my supervisors, Prof. Gabriel A. Dedeke and Dr. Opeyemi I. Ayanda, for the intellectual expertise and contributions made toward the success of this research. Thank you both for being more than academic advisors and mentors. You became fathers who wanted the best out of a child. Thank you for your guidance and laboratory visits sirs.

I am also grateful to members of the Departmental Postgraduate Committee; Dr. Eze F. Ahuekwe and Dr. Rotimi S. Aworunse and the erstwhile Departmental Postgraduate Coordinator, Dr. Olayemi O. Akinnola for their constructive criticisms and mentoring styles.

My gratitude also goes to my father, Surv. Oyebanji Iyanda Akinhanmi, for the financial, moral, and spiritual support till the end of this programme. Thank God you are alive and well to witness this feat that you saw many years ago. Thank you very much, sir.

To my late mother, Mofolorunsho Omowunmi Margret Akinhanmi, you saw it coming too but not here to witness it. I am grateful to you; your words of encouragement lived through and geared me on.

My profound gratitude goes to my siblings; Mr. Akinfolarin Akinhanmi, Mrs. Mojisola Egbeyemi, and Mrs. Temilola Obademi, and to my ever-supportive brother-in-law; Mr. Olakunle Egbeyemi, my “Thank you” is finally here. Thank you all for your spiritual, moral, and financial support. To my husband, Mr. Akintayo Beckley, may God honor you and reward your selflessness; thank you. I am grateful for the gift of my son, Oluwafunmishe TiOluwanimi Beckley, thank you for giving me peace and cooperation during the programme. I also appreciate Mr. and Dr. Mrs. Olawande, for their financial and moral supports. My gratitude also goes to my dear Aunty; Faith Ayeotan. Thank you for the moral and financial supports. God bless you all.

I thank all members of the faculty and staff of the Department of Biological Sciences, for rendering assistance when needed; particularly, Mr. Gbenga Taiwo, Dr. Adetutu Bello, Dr. Rotimi Aworunse, Mrs. Ibukun Ajiboye, Mrs. Bose Adekeye, Mr. Oba Micheal, and Dr. Margaret Oniha. May God bless you all. My gratitude also goes to Mr. Adeyemi and Miss Bose of the Department of Biochemistry. Thank you very much for your availability, technical assistance, and insightful contributions. My appreciation also goes to Prof. Nsikak U. Benson and Dr. Omowunmi Fred-Ahmadu, my informal mentors; your insightful advice and availability were of great value. Lastly, my appreciation goes to Mr. Joseph Anite, MD Etina Environmental Services, thank you for the technical, instrumental and human resource assistance. I am grateful.

TABLE OF CONTENTS

LIST OF TABLES

TABLES **TITLE OF TABLES** **PAGES**

LIST OF PLATES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AChE – Acetylcholinesterase

A549 cell – Adenocarcinoma human alveolar basal epithelial cell

AST – Aspartate aminotransferase

ALT – Alanine aminotransferase

BCF – Bioconcentration factor

BD – Badagry

BSAF – Biota-sediment accumulation factor

℃ - Degree Celsius

CG – *Clarias gariepinus*

CN – *Chrysichthys nigrodigitatus*

CYP 1A – Cytochrome P450 gene

EP – Epe

FTIR – Fourier Transform Infrared Spectroscopy

GN – *Gymnarchus niloticus*

h – hours

HCl – Hydrochloric acid

HDPE – High-density polyethylene

HNO_3 – Nitric acid

H_2O_2 – Hydrogen peroxide

HSP 70 – Heat shock protein gene

KOH – Potassium hydroxide

LDPE – Low-density polyethylene

MK – Makoko

mg/L – Milligram per litre

min – Minutes

M cell – Microfold cell

MP(s) – Microplastic(s)

MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

NADH – Nicotineamide adenine dinucleotide

NaOH – Sodium hydroxide

ng/g – Nanogram per gram

nm - Nanometre

ON – *Oreochromis niloticus*

PA – Polyamide

PAHs – Polycyclic Aromatic Hydrocarbons

PCR – Polymerase Chain Reaction

PE – Polyethylene

PET – Polyethylene terephthalate

POPs – Persistent Organic Pollutants

PP – Polypropylene

PVC – Polyvinyl chloride

PVA – Polyvinyl alcohol

PS – Polystyrene

Py-GC-MS – Pyrolysis gas chromatography-mass spectroscopy

ROS – Reactive oxygen species

rpm – Revolutions per minute

SEM – Scanning electron microscope

STM – Scanning tunneling microscope

SK – SagboKoji

TDS-GC-MS – Thermal desorption gas chromatography mass spectroscopy

TEM – Transmission electron microscope

s – Seconds

ABSTRACT

Microplastics (MPs) are ubiquitous in the environment and have been a source of scientific concern. The detection of microplastics in edible aquatic species and the studies that highlight subsequent potential toxic effects under field conditions are few. Hence, this study determined the occurrence of microplastics and assessed their biological effect on selected commercial fish species in the Lagos Lagoon. Composite sampling technique was employed in retrieving surface water and benthic sediment samples from four locations; Badagry, Makoko, SagboKoji, and Epe, while selected fish species were procured directly from local fishermen and assessed for microplastic contamination. Thirty-two samples each of *Chrysichthys nigrodigitatus, Oreochromis niloticus, Gymnarchus niloticus* and *Clarias gariepinus* were analysed for microplastic contamination, oxidative stress, histopathology, and relative gene expression profile. Microplastics were observed and counted using a stereo microscope and plastic polymers were identified with Fourier-transform infrared spectroscopy. The gills, stomach, and liver were assessed for histopathological damage and oxidative stress using antioxidant enzyme biomarker; superoxide dismutase, glutathione, glutathione-S-transferase, and lipid peroxidation product, malondialdehyde. The relative gene expression profile of the fish liver was determined by the Real-time PCR analysis, with cytochrome 1a and heat shock protein 70 as the target genes, and β-actin as the reference gene. The seasonal variations in microplastic load and effects were determined in the sampling locations for inference. Statistical significance was established at $p \leq 0.05$ between the abundance of microplastics across all the sampled locations. In the dry season, microplastic abundance was significantly high in Badagry at 71.4 ± 6.2 particles/L, and 3241.5±1069.5 particles/kg in the surface water and sediment respectively, whereas the fish species from Epe recorded the maximum microplastic presence at 34.7±9.4 particles/fish. In the rainy season, the microplastic load was predominant in the surface water and sediment from Badagry at 130.9 ± 7.0 particles/L and 5946.3 ± 543.7 particles/kg respectively, while high microplastic load (98.9 ± 35.8 particles/fish) was recorded in the fishes from Epe. *Oreochromis niloticus* accumulated the highest number of microplastics (38.2 particles/fish) in the dry season while *Gymnarchus niloticus* accumulated more (101.8 particles/fish) in the rainy season. Fibre was the predominant microplastic shape at 83% in surface water, 54% in sediment, and 61% in fish, with polyethylene, polychloroprene, and polyvinyl alcohol being the pre-eminent plastic polymer found. SOD activity and MDA levels were significantly higher in MP-laden fish tissues than in MP-free samples across both seasons ($p \leq 0.05$). Stunted gill lamellae and degenerated necrotic hepatocytes were observed in the gill and liver of microplastic-contaminated fishes, respectively, while the stomach tissues showed no visible lesion. Cytochrome P450 gene was up-regulated as against the housekeeping β-actin gene in the contaminated fish liver. The microplastic load was significantly ($p \leq 0.05$) higher in the rainy season than in the dry season. Biomarker responses in the fish species were also statistically higher in the rainy season than in the dry season. This study establishes an association between microplastic load in fish tissues and toxicological effects, calling for urgent measures to check microplastic contamination. However, further investigation is necessary to elucidate the risks and impact of chronic microplastic exposure on public health via the consumption of microplastic-contaminated fishes.

Keywords: Clarias gariepinus, gene expression, Lagos lagoon, microplastics, Oreochromis niloticus, oxidative stress