

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

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(20PCO02218)**

JUNE, 2024

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BY

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**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES
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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

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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.

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DEDICATION

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

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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

°C - 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

HNO₃ – Nitric acid

H₂O₂ – 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