ORIGINAL PAPER

https://dx.doi.org/10.4314/njpr.v21i1.5

Nig. J. Pharm. Res. 2025, 21(1) pp 43-49
ISSN 0189-8434 e-ISSN 2635-3555

Analysis of Antibiotic and Heavy Metal Residues in *Clarias gariepinus* (African Catfish)

A. M. OGUNNOWO [A-F]*, A. F. POPOOLA[A-D], O. P. ASANGA[C-E], O. O. AYANLEYE[C-E], B. O. OYAWALUJA[C-F], A. A. ADEPOJU-BELLO[A-F]

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Pharmaceuticals, including antibiotics and heavy metals, are emerging contaminants in many African countries affecting all facets of life as a result of indiscriminate disposal.

Objectives: This study evaluates the presence and potential health risks of pharmaceuticals in catfish from western Nigeria.

Material and Methods: Ten Fish samples were collected from local markets and aquaculture farms. Antibiotic and heavy metal residues were analysed using solid-phase extraction (SPE) and high-performance liquid chromatography (HPLC) and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) respectively.

Results: Seven antibiotics were tested: Trimethoprim (TM), Tetracycline (TC), Ciprofloxacin (CP), Sulfamethoxazole (SZ), Enrofloxacin (EN), Metronidazole (MT), and Ampicillin (AP). TM, TC, CP, SZ, EN, and AP were detected. TC was found at 0.2 µg/g, exceeding the maximum residue limits (MRLs). CP, SZ, EN, and AP averaged 0.1 µg/g, also above MRLs. TM was below MRLs at 0.05 µg/g, and MT was absent in all samples. On the other hand, the levels of Iron, Zinc, and Chromium present in all samples ranged from 6.0196 to 846.8667 mg/kg, 0.3985 to 579.4926 mg/kg and 0.2336 to 18.8947 mg/kg respectively, well above the EU's maximum safe level of 5 mg/kg. Arsenic, Manganese Copper, Cobalt, Arsenic have concentrations below the maximum safe level. All samples were Lead (Pb)-free which conforms to the EU specification.

Conclusion: The findings confirm that antibiotics and heavy metals are significant contaminants in catfish. This highlights the urgent need for legislation to control pharmaceutical waste, regulate antibiotic use in aquaculture, and implement stringent screening and monitoring measures to safeguard public and aquatic life.

Keywords: *Clarias garipienus*; Antibiotics; Heavy metals contaminations; Pharmaceutical waste

INTRODUCTION

The global aquaculture industry has experienced remarkable growth over the past two decades, with production increasing by 62.2% from 45.4 million tonnes in 2004 to 73.8 million tonnes in 2014, now accounting for 44% of total fish production worldwide (FAO, 2014, 2016). This rapid expansion has been driven by increasing demand for seafood, advancements in aquaculture technology, and the need for sustainable protein sources. However, this growth is accompanied by concerns regarding antibiotic misuse, heavy metal contamination, and environmental sustainability.
Antibiotics, once hailed as a 20th-century wonder discovery (Inda-Díaz et al., 2023; Radtke, 2023), are frequently prescribed but often misused (Shao *et. al.,* 2021), driving the emergence and spread of

antibiotic resistance (Ruiz J., 2021). This resistance poses a significant threat to patient care globally (Aijaz *et. al.*, 2023), with an estimated 4.95 million deaths attributed to bacterial antimicrobial resistance (AMR) in 2019, disproportionately affecting western sub-Saharan Africa (Murray C.J.L, 2022).

In Nigeria, studies have detected antibiotic residues in fish and seafood (Ogunrinu et al., 2017; Adeyemi et al., 2018), while heavy metals from anthropogenic activities contaminate aquatic environments, harming aquatic life and posing environmental risks (Mishra *et. al*., 2019; Sonone *et. al*., 2020). Heavy metal exposure can lead to neurotoxicity, carcinogenicity, and other health issues (ATSDR, 2020), with research indicating contamination in fish from Nigerian markets (Olajire et al., 2011; Oyewale et al., 2017).

The Nigerian aquaculture industry faces unique challenges, including inadequate regulation, poor farming practices, and limited access to sustainable feed and technology (Sadan & Amuda, 2023). These factors contribute to the misuse of antibiotics and heavy metals, compromising the safety and quality of aquatic products. Effective monitoring and research programs are crucial to address these concerns and prevent toxic effects on aquatic organisms and humans through the food chain. This study aims to examine the current state of antibiotic resistance and heavy metal contamination in Nigerian aquaculture, highlighting the need for improved regulation, sustainable practices, and public awareness.

METHODOLOGY

Study Area and Sample Collection

This study was conducted in the Western region of Nigeria, specifically focusing on four fish markets (Akoka, Yaba, Ojota, and Mowe) and two rivers in Ogun State. A total of ten Catfish (*Clarias Gariepinus*) samples were randomly collected from these locations between June and July 2021. The samples consisted of five dried and five fresh fish, obtained from both market and river sources.

Sample Preparation

Fish muscle tissues were carefully removed using a stainless-steel knife, homogenized to ensure uniformity, and stored in sterile containers for subsequent analysis. Smoked fish samples were pulverized using a mortar and pestle to facilitate extraction.

Antibiotic Residue Analysis

Sample Extraction

Approximately 5g of blended sample was transferred into a 100ml volumetric flask, and 50ml of McIlvaine-Na_2EDTA extraction solvent was added. The mixture was vigorously agitated for 1 hour and centrifuged at 4000 rpm for 10 minutes at 4°C.

Solid Phase Extraction (SPE) Purification and Cleanup

The extract was loaded onto an Agilent BondElut C18 cartridge, previously activated with 6ml of methanol and conditioned with 6ml of extraction solvent. The cartridge was washed with 6ml of distilled water and eluted with 3ml of methanol. The eluate was pre concentrated to 1ml using a stream of nitrogen gas.

High-Performance Liquid Chromatography (HPLC)

HPLC conditions: Flow rate: 1.0 ml/min, Column: C18, Detector: Multiple wavelength UV detection (225 nm and 280 nm), Injection volume: 20 µl, Mobile Phase A: 20mM NaH2PO4 (pH 3.0), Mobile Phase B: Acetonitrile (HPLC Grade), Gradient elution: 0-3 minutes (10% B), 3-20 minutes (linear increase to 30% B)

Preparation of Standard Solution

Calibration curves were prepared for each antibiotic (Tetracycline, Ciprofloxacin, Ampicillin, Enroflaxacin, Trimethoprim, Sulfamethoxazole and Metronidazole using serial dilutions of stock solutions (100 ppm). A range of concentrations (0.1-20 ppm) was prepared for each antibiotic.

Heavy Metal Analysis

Sample Preparation

Heavy metal analysis of fish samples involves a multi-step digestion and preparation process.

Digestion, Dilution and Filtration

Approximately 5g of homogenized fish sample is weighed into a digestion flask. 10ml of concentrated HNO_3 (65-70%) is added, and the mixture is digested on a Q-block automatic digester or hot plate at 180°C for 1 hour, or until the brown fumes become colorless. After cooling, the sample is diluted to 25ml with purified water and filtered through a 0.45µm filter paper to remove particulate matter.

Acidification and Storage

A 1mL of concentrated HNO_3 is added to the filtered sample to ensure stability. The acidified sample is stored in a clean, sterile container and refrigerated at 4°C until analysis.

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) Analysis

ICP-OES analysis measures the concentrations of heavy metals, including: As, Cd, Co, Cr, Cu, Fe, Mn, Pb, and Zn

Preparation of Calibration Standard Solutions

Calibration standard solutions were prepared from stock solutions (1000 µg/mL) of individual heavy metal standards (As, Cd, Co, Cr, Cu, Fe, Mn, Pb, and Zn).

Preparation of Working Standard Solution

A 10 mL aliquot of each stock solution was transferred into a 100 mL volumetric flask and diluted to volume with distilled water, yielding a working standard solution with a concentration of 100 µg/mL (Solution A).

Preparation of Calibration Standards

Calibration standards were prepared by diluting Solution A with distilled water to achieve six concentration levels: 0.5 mg/L, 1.0 mg/L, 2.0 mg/L, 5.0 mg/L, 10.0 mg/L, 20.0 mg/L. These calibration standards were used for Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) analysis.

Method Validation

Reference materials were used for analytical method validation. Blank analysis and individual reference standards were employed to ensure accuracy and precision.

Statistical Analysis

Arithmetic mean and standard deviation were calculated for positive quantifiable samples.

RESULTS

The analysis of antibiotic residues in fish samples revealed varying levels of contamination. A total of 10 fish samples, comprising 5 dried and 5 fresh samples, were collected from four markets (Akoka, Yaba, Ojota, and Mowe) and two rivers (Mowe and Ota) in Ogun State, Nigeria. The samples were analyzed for residues of nine antibiotics (Metronidazole, Ampicillin, Trimethoprim, Tetracycline, Ciprofloxacin, Enrofloxacin, Sulfamethoxazole) using High-Performance Liquid Chromatography (HPLC). Additionally, heavy metal analysis was conducted using ICP-OES to determine the concentrations of As, Cd, Co, Cr, Cu, Fe, Mn, Pb, and Zn.

The limits of detection (LOD), limits of quantification (LOQ), residual standard deviation, and regression data for the antibiotic analysis are presented in Table 1. Calibration data for the antibiotic reference standards are shown in Table 2. The concentrations of antibiotic residues and heavy metals in the test samples are reported in Tables 3 and 4, respectively, while figure 1 showed mean average concentration (mg/kg) of antibiotics in fish samples, and figure 2 showed the mean concentration of heavy metals in fish samples.

Table 1: Limit of detection (LOD), limit of quantification (LOQ), residual standard deviation (RSD), regression (r^2) and maximum residual limits (MRLs)

Antibiotics	LOD	LOQ	RSD	R^2	MRLs	frequency
Metronidazole	0.214	0.715	1.95481	0.99999	Prohibited	ND
Ampicillin	0.248	0.825	1.34048	0.99999	0.050	90
Trimethoprim	0.572	1.906	21.32605	0.99993	0.100	100
Tetracycline	0.243	0.811	1.43292	1.00000	0.100	100
Ciprofloxacin	0.176	0.588	7.39466	1.00000	0.100	100
Enrofloxacin	0.222	0.738	10.50469	0.99999	0.100	100
Sulfamethoxazole	0.325	1.085	7.21929	0.99998	0.100	100

Table 2: Calibration data for antibiotics reference standard

Concentration	Peak Area (MAU)						
(ppm)	MT	TM	TC	CP	EN	SM	AP
0.1	3.130	13.70249	_	13.1535	12.3153	9.99802	1.6928
0.5	13.67	54.41705	6.64863	50.16417	53.1375	35.0732	7.4899
1.0	27.57	110.1951	14.5313	114.5788	121.6202	71.36481	15.418
2.0	53.04	231.59953	32.13696	232.20413	260.0748	126.83037	30.50669
5.0	135.1	559.79651	84.46629	613.0988	1406.709	324.26645	78.9831
10	268.3	1176.9704	175.3575	1248.358	2827.367	650.81042	158.717
20	546.95	2236.6384	350.4345	2504.260	2827.36	1332.802	324.200

**Trimethoprim (TM), Tetracycline (TC), Ciprofloxacin (CP), Sulfamethoxazole (SZ), Enrofloxacin (EN), Metronidazole (MT), and Ampicillin (AP)*

Table 3: Concentration (mg/kg) of antibiotics in fish samples

Sample	TC	CP	EN	SM	MT	AM	TM
A	0.21	0.31	0.13	0.55	0.0	0.16	0.29
B	0.17	0.22	0.05	0.07	0.0	0.34	0.16
C	0.12	0.26	0.05	0.11	0.0	0.37	0.04
D	0.19	0.06	0.22	0.44	0.0	0.0	0.04
E	0.16	0.38	0.04	0.05	0.0	0.53	0.03
F	0.39	0.14	0.12	0.11	0.0	1.02	0.04
G	0.13	0.23	0.04	0.18	0.0	0.66	0.09
H	0.24	0.13	0.04	0.07	0.0	0.50	0.04
I	0.56	0.16	0.08	0.38	0.0	0.21	0.07
J	0.45	0.04	0.15	0.68	0.0	3.35	0.09

**Trimethoprim (TM), Tetracycline (TC), Ciprofloxacin (CP), Sulfamethoxazole (SZ), Enrofloxacin (EN), Metronidazole (MT), and Ampicillin (AP)*

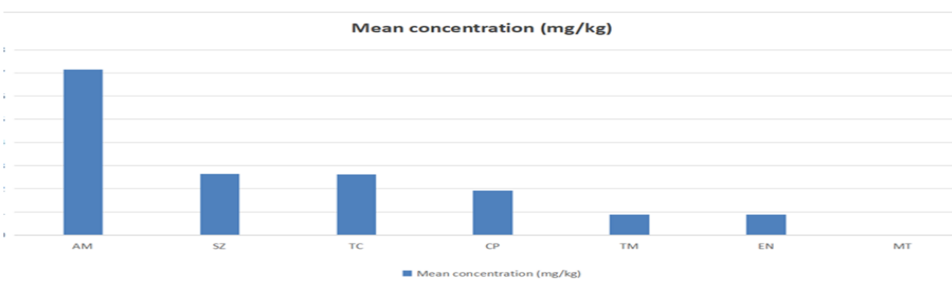

Figure 1: Mean Average concentration (mg/kg) of antibiotics in fish samples

Table 4: Concentration (mg/kg) of heavy metals in the fish samples

Sample	As	Cd	Co	Cr	Cu	Fe	Mn	Pb	Zn
A	0.4992	3.2941	1.5505	0.2336	1.9467	6.9109	0.0	0.0	4.5035
B	0.3410	0.2937	0.0	0.8313	1.6125	6.8422	0.0	0.0	0.3985
C	0.4815	0.0	0.0	0.6460	1.7487	6.0196	2.6418	0.0	2.8185
D	0.0	0.0	0.0	0.0	1.9467	846.667	0.0	0.0	491.2173
E	0.8939	0.0	0.0	0.0	2.2035	8.2504	0.0	0.0	5.5185
F	0.0	0.0	0.0	0.0	94.0946	258.8198	0.0	0.0	53.3122
G	0.0	0.0	0.0	1.3988	110.0565	271.8876	0.0	0.0	127.3931
H	0.0	0.0	0.0	18.8947	156.5153	285.6005	0.0	0.0	579.4926
I	0.0	0.0	0.0	0.0	108.0381	209.6785	0.0	0.0	110.7486
J	0.0	0.0	0.0	0.0	88.7703	170.9338	0.0	0.0	155.7186

A - Ota market dry B - Ojota market dry C - Ifo market dry D - Akoka market dry E - Mowe market dry F - Mowe River G - Ota River H - Mowe market Fresh I - Ojota market Fresh J - Yaba market Fresh

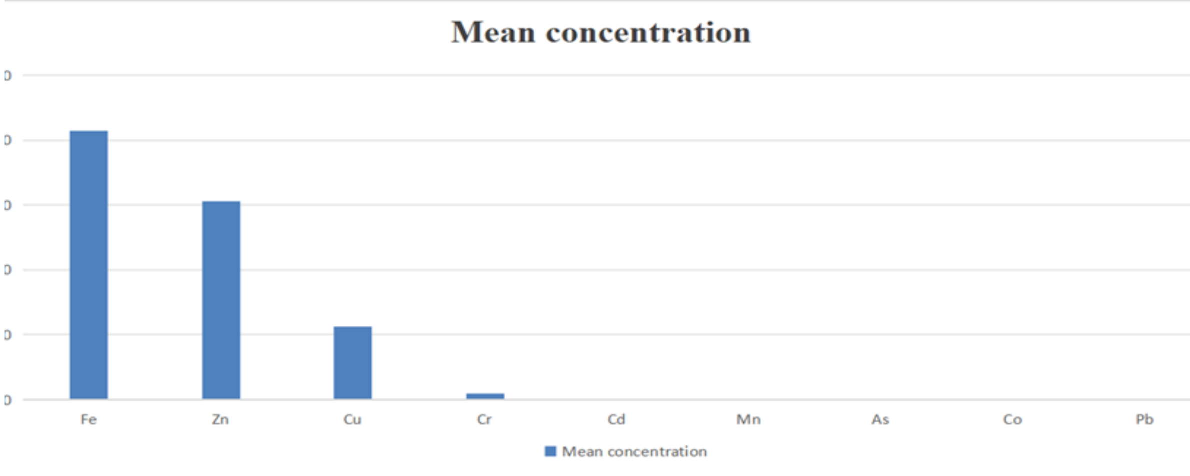

FIGURE 2: Mean concentration of heavy metals in fish samples

DISCUSSION

The presence of antibiotic residues in fish samples is a concern due to the potential development of antibiotic-resistant bacteria and harmful effects on human health. The analysis of antibiotic residues in fish samples revealed varying levels of contamination. The results showed that some fish samples exceeded the maximum residual limits (MRLs) set by regulatory agencies, posing potential health risks to consumers.
Metronidazole, Ampicillin, Trimethoprim, Tetracycline, Ciprofloxacin, Enrofloxacin, and Sulfamethoxazole were detected in various samples, with concentrations ranging from 0.00 to 3.35 mg/kg (Table 3).
The results showed that some samples exceeded the MRLs (table 1) for certain antibiotics while some were not detected. For instance, Metronidazole was not in all samples. Ampicillin, Trimethoprim, and Tetracycline exceeded their respective MRLs in several samples. This is alarming, as excessive antibiotic residues can lead to adverse health effects, including allergic reactions, liver damage, and increased risk of antibiotic resistance.
The mean average concentration (mg/kg) of antibiotics in fish samples (figure 1) showed that Ampicillin has the highest concentration in the samples followed by Sulfamethoxazole and then Tetracycline.
The analysis of heavy metals revealed significant levels of Cd, Co, Cr, Cu, Fe, Mn, Pb, and Zn in some samples (table 4). The concentrations of these metals exceeded the recommended limits in some cases. Chronic consumption of heavy metal-contaminated fish can lead to serious health problems, including kidney damage, neurological disorders, and cancer.
The mean concentration of heavy metals in fish samples (figure 2) showed Iron has the highest abundance in the samples followed by Zinc and then Copper.
The presence of antibiotic residues and heavy metals in fish samples raises concerns about the safety of seafood consumption. Prolonged consumption of contaminated fish can lead to adverse health effects, including antibiotic resistance, liver damage, kidney damage, and increased risk of cancer.

CONCLUSION

The analysis of fish samples from Nigerian markets and rivers revealed alarming levels of contamination, posing significant risks to consumer health and food safety. Heavy metals like Arsenic, Cadmium, and Chromium were detected, while antibiotics like Ampicillin, Trimethoprim, and Tetracycline exceeded the MRL. These findings highlight widespread environmental pollution, noncompliance with regulations, and improper antibiotic use in aquaculture, contributing to residue contamination. To address these issues, immediate action is necessary. Regular monitoring, environmental cleanup initiatives, and strengthened regulatory enforcement are crucial. Compliance with recommended limits and guidelines must be ensured to protect public health. Educating stakeholders on safe practices and promoting responsible antibiotic use will safeguard public health, prevent antibiotic resistance, and protect the environment.

Government agencies, regulatory bodies, and stakeholders must prioritize food safety and environmental protection to ensure a healthier environment for future generations. Collaboration among stakeholders is vital to address contamination and ensure safe and sustainable seafood production.

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*Address for correspondence: Ogunnowo, Ayodeji Mopelola
Department of Pharmaceutical Chemistry,
Faculty of Pharmacy,
University of Lagos,
Lagos, Nigeria
Telephone: +2348140111791
E-mails: ogunnowo.ayodeji@yahoo.com

Conflict of Interest: None declared
Received: September 30, 2024
Accepted: February 4, 2025