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Levels of Antibiotics in Fish Species from Eleyele River, Ibadan, Nigeria

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Abstract

Antibiotics are commonly used to treat infections in humans and animals, and in aquaculture to prevent bacterial diseases. However, their excessive use in livestock and poultry have led to environmental contamination, particularly in water bodies, raising concerns about food safety and public health. This study investigated the presence of tetracycline, chloramphenicol, and metronidazole in three fish species—*Parachanna obscura* (Obscure snakehead), *Clarias gariepinus* (African sharp-tooth catfish), and *Oreochromis niloticus* (Nile tilapia)—from Eleyele River in Ibadan, Nigeria. Using solid-phase extraction and silica gel clean-up, followed by high-performance liquid chromatography with diode-array detection (HPLC-DAD), antibiotic residues were detected at concentrations ranging from below detection limits to 1311.82 ng/g. Method validation showed high sensitivity, with limits of detection (LOD) between 0.13–9.17 ng/g and limits of quantification (LOQ) from 0.41–27.78 ng/g. *P. obscura* and *C. gariepinus* had significant levels of all three antibiotics, while no residues were found in *O. niloticus*. The concentrations found exceeded the 0.1µg/g maximum residue limit set by the European Union, indicating a potential health risk for consumers and environmental harm. This study concluded that the fishes from Eleyele River were contaminated with antibiotic residues at levels above international safety standards. It is recommended that routine monitoring be enforced, alongside stricter regulations and public education on responsible antibiotic use in agriculture and aquaculture, to mitigate further contamination and protect public health.

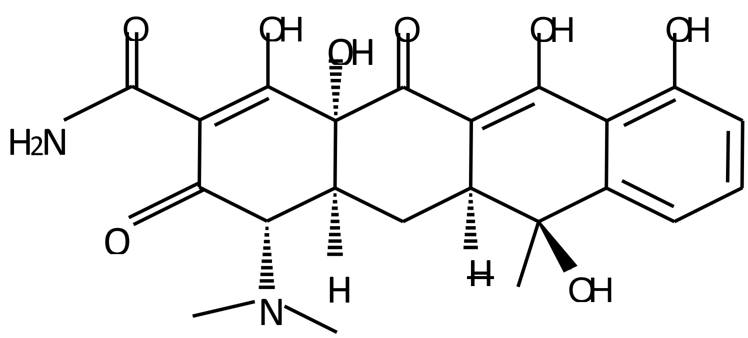
Keywords: antibiotics, fish contamination, Eleyele River, HPLC-DAD, food safety.

Introduction

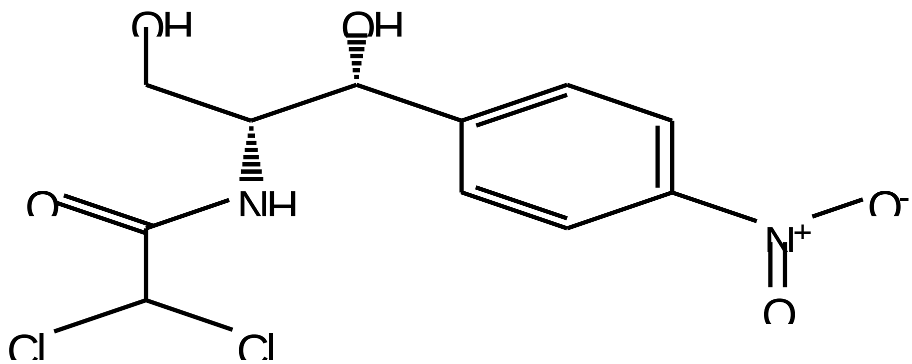
Eleyele River is a well-known for fish farming in Ibadan, Nigeria, its water is used for different activities which include domestic water supply, flood control, agricultural purposes, tourist [1-2]. The inhabitants are majorly Yoruba speakers and their main occupations are farming and fishing [3]. Pharmaceutical pollutants and their metabolites occur in both terrestrial and aquatic environments [4]. Antibiotics are used as antibacterial drugs, for aquaculture, livestock farming, human medicine which sometimes are misused, overused and released indiscriminately in form of effluent, excreta and sewage sludge [5]. Antibiotic residues in food can lead to antibiotic resistance, posing serious health threats to consumers and diminishing the effectiveness of antibiotics in treating infections [6-9]. Antibiotics are commonly used in fish farming to prevent and treat diseases, but their overuse can lead to the development of antibiotic-resistant bacteria and environmental pollution [10]. By assessing antibiotic residues in fish, fish farmers can evaluate the impact of antibiotic use in aquaculture and develop strategies to promote sustainable and responsible aquaculture practices [11].

Hence, determining the concentrations of antibiotic residues in fish is essential for safeguarding public health, ensuring compliance with regulations, and promoting sustainable aquaculture practices [12]. Anthropogenic factors influence the presence of excess antibiotics in river water and fish tissues through: Agricultural practices, human Medicine, Sewage sludge, industrial effluent and so on [13]. In Agricultural practices, Agriculture has played a vital role in the production of food for human consumption. [14]. However, agricultural practices such as livestock farming which involves raising of farm animals either for their meat, milk, hide etc. have been considered as one of the widest antibiotics markets [15-17]. Animal feeds, meals and rations are mixed together with antibiotics product for the treatment of bacterial infections, growth promotion or for the sake of its therapeutic application [18]. Remnants of these feeds, mixes with soil, rain falls on them, percolates into the soil and are carried away into the river or ponds through surface run off, erosion or underground water movement [19].

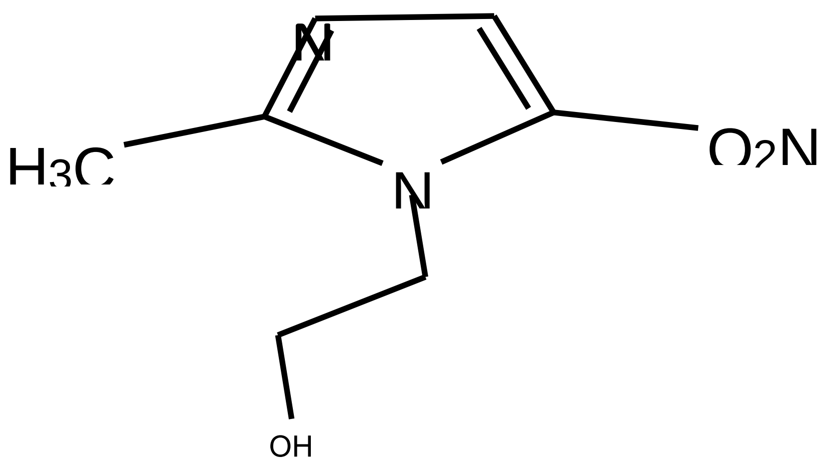
The structures of the investigated analytes of interest are given in Figure1.The compounds of these pharmaceuticals include tetracycline, chloramphenicol, and metronidazole.



Tetracycline



Chloramphenicol



Metronidazole

Figure 1: Structures of Some of the studied Polyaromatic Hydrocarbons [20].

In Human Medicine, bacterial infection is one of the diseases that attacks human. Apart from inhibiting these diseases, Antibiotics are used to prevent its spread and reproduction. It has been commonly observed, that after a bacterially infected patient has been prescribed an antibiotic drug or medication by a pharmacist, most patient do not always take the number of daily dosages as prescribed [21]. Once they have a prior feeling of relieve, they neglect the rest of the drug and most times dispose or discharge the remnants into the neighboring environment which seeps into water course [22]. The solute antibiotics dissolves into the river and constitute pollution. Chloramphenicol is a broad-spectrum bacterial antibiotic used against conjunctivitis, meningitis, plague, cholera, and typhoid fever [23]. As a consequence, chloramphenicol ends up polluting the aquatic environment. These drugs are sometimes under used, overused and in some cases abused.

Undigested form of the overused drugs is deposited in urine and faeces of both plant and Animals [24]. The sewage effluent is discharged improperly through pipe outlets, leaking septic system into flowing river water and ground water. Sewage sludge is categorized as one of the emerging organic pollutants, they are effluents containing different toxic substances including endocrine disruptors, pathogenic bacteria and heavy metals [25]. Although during sewage treatment, a fraction of the antibiotics is biodegraded but a larger percent of it is tightly bound and are absorbed by sewage sludge [26]. Effluents of industrial waste water or debris blown into water ways from land contaminated with industrial antibiotics-waste leach into the river and increases the concentration of antibiotics absorbed into the cells of aquatic microorganisms which in turn finds their way into the tissues of fish [27].

Several studies have been reported globally on antibiotics in terrestrial animals but there is dearth of information on their bioaccumulation in fish species especially in Nigeria. Therefore, this study intends to fill the gap. This study aimed to validate the analytical method for the determination of selected antibiotics (tetracycline, chloramphenicol and metronidazole) and applied the validated method to real samples of fish species from Eleyele River, Ibadan. Nigeria.

Materials and Methods

Chemicals and Reagents

Analytical standards of all target antibiotics were of high purity and were purchased from Sigma Aldrich (Steinheim, Germany). Methanol, dichloromethane, acetonitrile, water, and formic acid (all of HPLC grade) were obtained from Fisher Scientific (Fairlawn, N.J, USA). Hydrochloric acid and tin granules were obtained from BDH (Poole London). Sodium phosphate dibasic heptahydrate ($Na_2HPO_4.7H_2O$), Sodium phosphate monobasic monohydrate ($NaH_2PO_4.H_2O$) and ethanol were purchased from Sigma Aldrich (Steinheim, Germany). Sodium hydroxide, 99.5% for the buffer was obtained from Chemsol Scientific (Saint Louis, MO, USA). The absorbent (Silical gel) and Sodium sulphate (NA_2SO_4) used to remove moisture from the organic extract were obtained from ACROS Organics.

Description of the study area

Eleyele River is a commercial center for fish farming, the river is situated in the city of Ibadan between latitudes 7020' -7025'N, and longitudes 3051-3056." The river has an altitude depth of 125m above the sea level, minimum temperature 24.50C and annual rainfall 1262.3mm and falls within Ido LGA of Oyo State Nigeria [28].

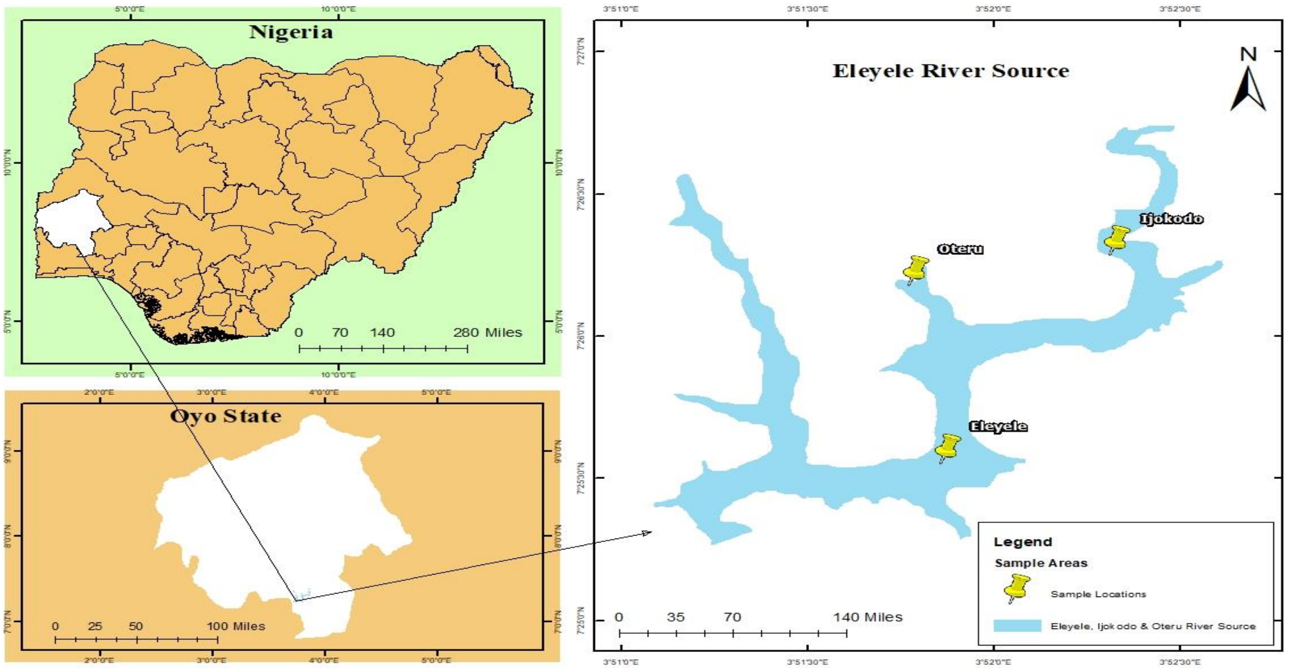


Figure 2: Map of the study area

Collection, Handling and Storage of the Fish Samples

Samples were collected randomly within a week and composited. The obtained samples were analyzed separately. Three (3) different sampling locations were sampled across Eleyele River. A total of forty-five fish samples of three different species of commonly consumed fish were caught with fish nets from Eleyele River, Ibadan at different points along the river (Iteru, Ijokodo and Eleyele). The three fish species were identified as: (Nile tilapia (*Oreochromis niloticus*) n = 15, African sharp tooth cat fish (*Clarias gariepinus*), n = 15, and Obscure snakehead fish (*Parachanna obscura*), n= 15. The choice of selection of the sampled fish species (Figure 2) were based on their nutritional values, affordability, and availability in season. The snake head fish for example is commonly consumed as wound healer while tilapia and cat fishes were recognized as the healthiest source of protein and omega-3 fatty acids.

The skin of the fish samples was removed and the muscle tissues were homogenized with a stainless-steel metal grinder. The mixing was repeated until the composition appeared to be homogenized and kept frozen until extraction [29-30].

Extraction Antibiotics from Fish Species

The fish species were extracted with a solid phase extraction method. Two grammes of each fish samples were weighed into 50mL centrifuge tube, 10 ml of phosphate buffer solution of pH 7 were added and the mixture allowed to stand for 15 minutes at room temperature. The solution is then vortexed for 1 minute, centrifuged at 3500rpm for 5 minutes [13,16,19,24]. The supernatant was transferred into another container. The process was repeated twice. The extracts were then combined together and concentrated to10 mL the cleanup process [31].

Cleanup of Extract

The extracts were cleaned with column chromatographic method [12,18,25,26.29]. The column was pre-conditioned with a mixture of both polar solvent (methanol) and non-polar solvent (Dichloro methane) 50/50 and packed with activated silical gel and Na_2SO_4(5:2) g with 3ml distilled water, after sample loading and washing, the analytes was eluted using 5 ml of the solvent mixture. The residues were dissolved in 500 µL of the mobile phase and subjected to HPLC-DAD for analysis after being filtered through 0.2 µm nylon syringe filter.

Optimized HPLC-DAD Conditions

The HPLC/DAD analyses weas conducted using an optimized High-Performance liquid Chromatograph hyphenated to a Diode Array Detector, Diode array detector (DAD) 254nm was used to obtain information over a wide range of wavelengths at a time to confirm the analyte identity at a time. HPLC-DAD instrument was chosen for the analysis because it is fast, sensitive and has high separation efficiency. Acetonitrile gradient with 0.1% formic acid (30:70) % and flow rate 0.7 ml/min was chosen and shown to give good separation and resolution of the analytes.

Results and Discussion

Method validation

Table 1 presents the critical validation parameters—Limit of Detection (LOD), Limit of Quantification (LOQ), and Coefficient of Determination (R)—for the developed HPLC-DAD method used in the quantification of tetracycline, chloramphenicol, and metronidazole in fish samples.

The Limit of Detection (LOD) represents the lowest concentration at which the presence of a compound can be reliably distinguished from the background noise, while the Limit of Quantification (LOQ) denotes the lowest concentration that can be quantitatively measured with acceptable precision and accuracy. Chloramphenicol showed the highest sensitivity, with an LOD of 0.13 ng/g and an LOQ of 0.41 ng/g. These exceptionally low values indicate that the method is highly capable of detecting and quantifying even minute traces of chloramphenicol in fish tissue. This is especially important given the stringent international regulations on chloramphenicol due to its carcinogenic and aplastic anemia-causing potential. Metronidazole had a moderate LOD of 6.69 ng/g and LOQ of 20.28 ng/g, suggesting that while the method is sensitive, it requires higher residue concentrations to achieve accurate quantification compared to chloramphenicol. Metronidazole, often used for treating protozoal and anaerobic bacterial infections, is also under regulatory scrutiny, which makes effective monitoring essential. Tetracycline had the highest LOD (9.17 ng/g) and LOQ (27.78 ng/g) among the three. While these values are higher, they still fall within an acceptable sensitivity range for environmental residue analysis. Tetracycline is one of the most commonly used antibiotics in aquaculture, so the method's ability to detect it at sub-microgram levels remains valuable for routine surveillance [32].

The coefficient of determination (R = 1) for all three antibiotics indicates perfect linearity across the calibration range. This means that the analytical response is directly proportional to the concentration of the analyte, allowing for accurate quantification. An R value of 1 reflects an ideal scenario in analytical chemistry and provides strong evidence of the method's reproducibility and consistency in detecting varying concentrations.

Table 1: Developed Method Validation Parameters

Antibiotics	LOD	LOQ	R
Tetracycline	9.17 ng/g	27.78 ng/g	1
Chloramphenicol	0.13 ng/g	0.41 ng/g	1
Metronidazole	6.69 ng/g	20.28 ng/g	1

LOD =Limit of detection, LOQ =Limit of quantification, R= Coefficients of Determination.

The high sensitivity of the method, particularly for chloramphenicol, ensures that even trace-level contaminations can be identified, which is crucial for meeting regulatory standards such as the European Union's maximum residue limits (MRLs) and the USDA's safety thresholds. For antibiotics like tetracycline and metronidazole, although the detection thresholds are higher, they are still sufficiently low to detect levels that may pose health risks, especially in contaminated environments. Additionally, the combination of low LODs/LOQs and perfect linearity supports the use of this method in both routine surveillance and regulatory compliance monitoring. The method is suitable for diverse fish species and can be applied to assess antibiotic contamination in different aquatic ecosystems [33].

Concentrations of antibiotic in Real Fish Species after Validation

Table 2 presents the concentrations of three antibiotics—chloramphenicol, metronidazole, and tetracycline—in the muscle tissues of three commonly consumed fish species: *Parachanna obscura* (Snakehead), *Clarias gariepinus* (Catfish), and *Oreochromis niloticus* (Tilapia).

Table 2 presents the concentrations of chloramphenicol, metronidazole and tetracycline antibiotics detected in the studied fish matrices. Chloramphenicol was determined, and the concentrations were highlighted as 0.35 µg/g in *Parachanna obscura* sample, 0.24 µg/g in cat fish while nothing was detected in Tilapia fish sample. The *Parachanna obscura* fish had the highest overall contamination, especially for tetracycline, which was detected at an extremely high concentration of 1311.82 µg/g—over 13,000 times the maximum residue limit (MRL) of 0.1 µg/g set by the European Union. This suggests a severe level of antibiotic exposure likely due to runoff from agricultural or aquaculture activities. The presence of both chloramphenicol and metronidazole above detection limits indicates multiple source contamination and raises concerns about bioaccumulation and ecosystem health.*Clarias gariepinus* also showed high levels of tetracycline (1179.11 µg/g), similar in magnitude to *Parachanna obscura*, which strongly suggests that both species were exposed to similar contamination sources, possibly due to their benthic feeding habits or overlapping habitats within the Eleyele River. Chloramphenicol was also detected above the MRL, while metronidazole was below detection limit (BDL), implying either a lower usage or a faster degradation rate of metronidazole in this species or part of the river.

The levels of chloramphenicol and tetracycline detected in both *Parachanna obscura* and *Clarias gariepinus* greatly exceed international safety limits, making their consumption potentially hazardous. Long-term exposure to such residues may lead to antibiotic resistance, allergic reactions, and toxicological effects in humans [34]. The data suggest unregulated or excessive use of antibiotics in nearby agricultural or aquaculture practices, which may be leaching into the Eleyele River. The complete absence of residues in tilapia shows that not all species are equally affected, highlighting the importance of species-specific monitoring and ecological risk assessment. In contrast to the other two species, no antibiotic residues were detected in *Oreochromis niloticus*. This may be attributed to several factors. *Oreochromis niloticus* is mostly herbivorous or omnivorous and feed near the surface or mid-water, potentially reducing their exposure to sediment-bound contaminants [35]. They may metabolize or eliminate antibiotics more efficiently [36]. They may also inhabit less polluted areas of the river [37]. The clean profile of *Oreochromis niloticus* positions it as the safest species for consumption among the three, based on this study. The trend in the contamination level is *Oreochromis niloticus* > *Clarias gariepinus* > *Parachanna obscura* (Table 2).

Table 2: Concentrations of antibiotic residues Eleyele River Fish species

S/N	Fish Species	Chloramphenicol(µg/g)	Metronidazole(µg/g)	Tetracycline(µg/g)
1	P. obscua	0.35	0.80	1311.82
2	C. gariepinus	0.24	BDL	1179.11
3	O. niloticus	BDL	BDL	BDL

BDL= Below Detection Limit

Additionally, a lower concentration of chloramphenicol at 0.24 µg/g determined in the African sharp tooth cat fish was different from the findings of William *et al.*,[38] where chloramphenicol activity could not be detected (< .5 ug/ml) in any of the three pooled cat fish specimens used. Ayman, *et al.*,[39]in his work on the effects of allium cepa and chloramphenicol on hematological parameters, histopathology and survival of catfish sub-adults infected with pseudomonas aeruginosa showed that chloramphenicol was present at the minimum inhibitory concentrations of 50 mg/mL in catfish and that it can be exploited for combatting infections of P. aeruginosa in fish. This study findings also indicated that chloramphenicol was below detection limit in tilapia fish sample but the findings of Shaodong *et al.*,[40] on rapid determination of chloramphenicol in tilapia using Ultra-high performance liquid chromatography revealed that Chloramphenicol was detected at 0.10ug/kg. The differences in the occurrence of antimicrobial residues in the present study and other reports might be due to the differences in the size of fish species, season of sampling, and their chosen extraction method. In this study, the concentration of tetracycline and metronidazole were reportedly higher than the maximum permissible limit set by the European Union 0.1 ppm and the USDA Foreign Agricultural Science of 2018. This confirmed the fact that antibiotics are being misused, overused and are released indiscriminately into water bodies in form of animal feed, sludge or excreta [41-45].

Globally, research had shown that the rate of mortality due to antibiotic drug resistance is higher in under developing and developing countries in Asia, Africa and South America [46-47]. Although the use of chloramphenicol in the treatment of bacterial diseases of fish has been banned in Nigeria yet, result showed from this study showed that chloramphenicol was discovered in both *Parachanna obscura* (0.35 µg/g) and *Clarias gariepinus* (0.24 µg/g) except from *Oreochromis niloticus*. This could be as a result of secret use or their being manufactured under different trade names despite their prohibition.

The levels of antibiotics obtained in this study differs from the findings reported by Adetunji et al., [48] where tetracycline level was between 3.077±0.538 ppm in tilapia and 1.9820±0.486 ppm in catfish while chloramphenicol was found between 1.108±0.300 ppm in tilapia and 0.822±0.223 in catfish. According to Watson, Metronidazole was discovered at 1.1 ± 0.1 ng/g in *Oreochromis niloticus*. The various concentrations of tetracycline, chloramphenicol and metronidazole residue obtained indicates that fish are potential source of antimicrobial contamination and could transfer antibacterial resistance to its consumers.

Concentrations of Antibiotics in the Mixed Samples

Concentrations of antibiotics in the mixed samples were investigated and the result obtained is as highlighted in table 3. The three Antibiotics were determined from the replicate of the mixed samples, the concentrations obtained were; tetracycline 1674.96 µg/g, chloramphenicol 1.31 µg/g and metronidazole 5.34 µg/g. The total obtained values were above the minimum required performance limit (0.3 µg/kg) set by European community [49] and therefore not safe for consumption. Also, Tetracycline was found to exceed the recommended consumption and absorption limit for fish (200ppm) therefore, the consumption of such contaminated fish might pose potential health risks to its consumers [50]. Their distribution trend in the mixed samples is: mixed sample 2 > mixed sample 3 > mixed sample 1 fish (Figure 3). Figures 4 and 5 gives the percentage distribution of the antibiotics in fish species and their representative chromatograms respectively.

Table 3: Concentrations of Antibiotics in mixed samples of fish

Replicate Mixed Samples	Tetracycline (µg/g)	Chloramphenicol (µg/g)	Metronidazole (µg/g)
Mixed Sample 1	1686.62	1.01	5.38
Mixed Sample 2	1662.57	1.76	5.08
Mixed Sample 3	1675.67	1.14	5.59
Mean	1674.96	1.31	5.34
Standard deviation	12.0	0.4	0.2
Relative Standard Deviation	0.7%	30.9%	4.4%

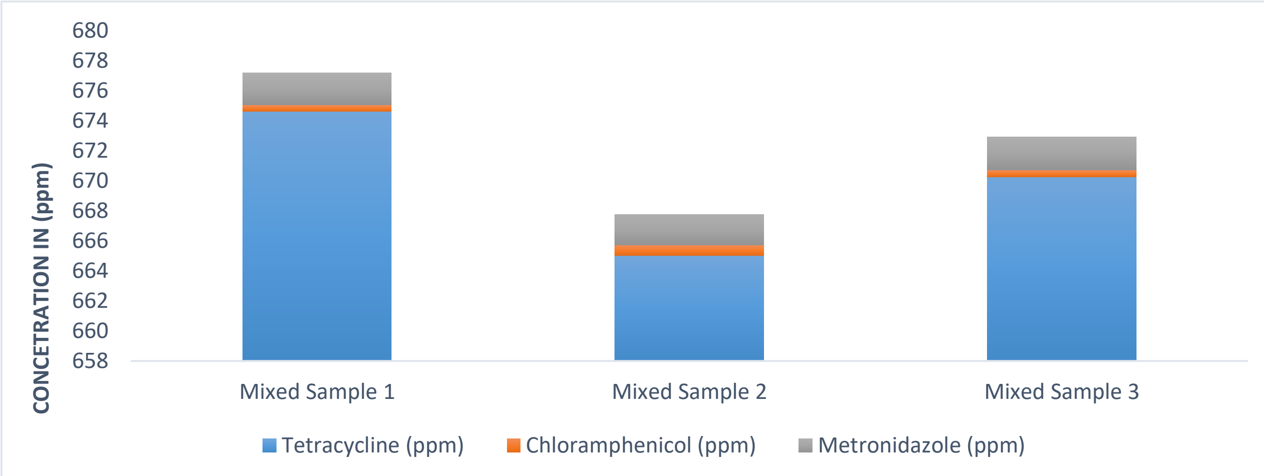


Figure 3: Levels of Antibiotics in the mixed sample of fish

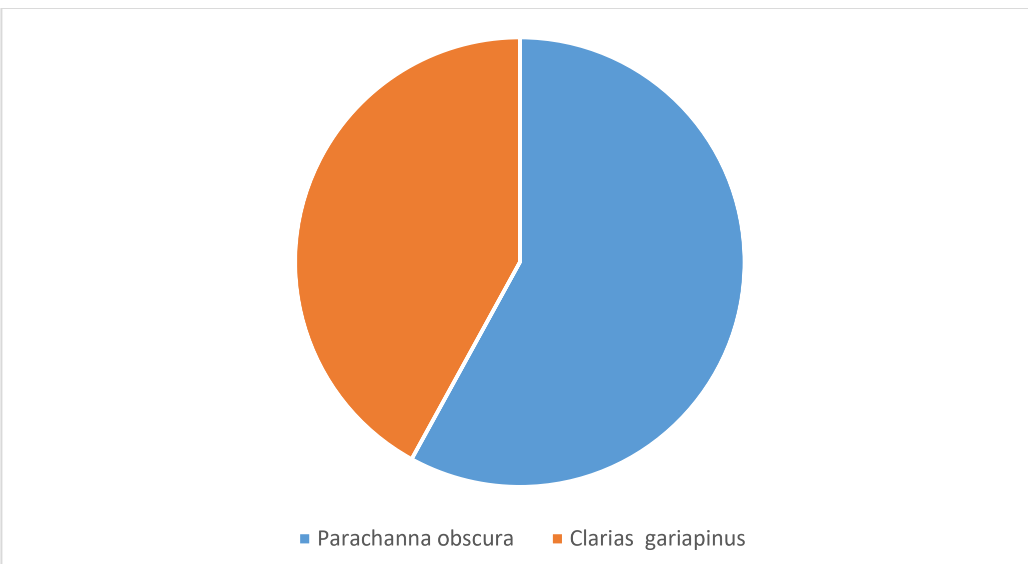


Figure 4: percentage distribution of the antibiotics in fish species

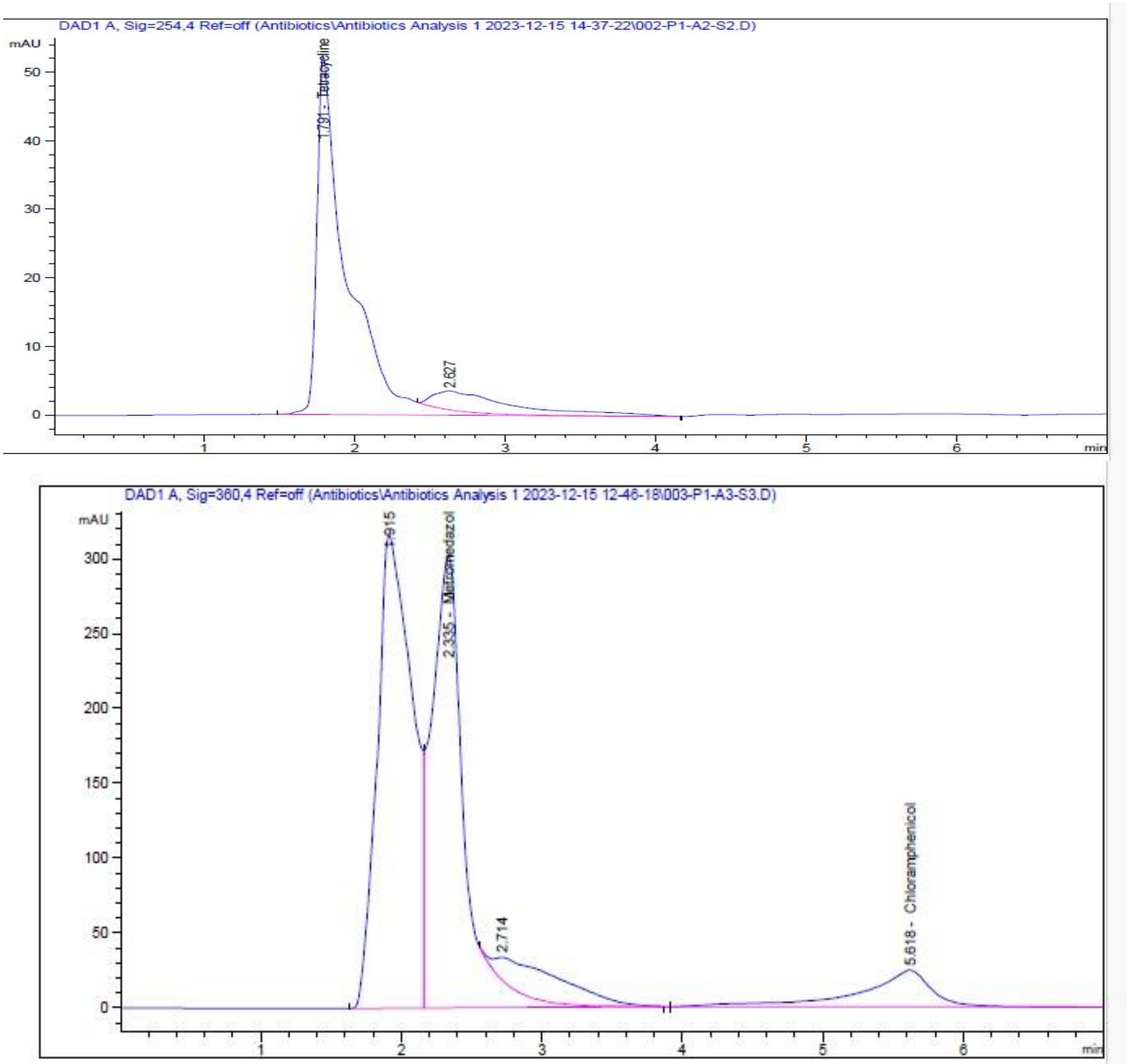


Figure 5: Representative Chromatograms of investigated Antibiotics in the Fish Species

Statistical Analysis

The statistical analysis indicated no significant difference between the means, as the accepted threshold for significance is a p-value of ≤ 0.05. Therefore, there is no significant relationship among the three analytes, suggesting that their presence in the fish samples are independent of one another.

Conclusion

This study confirmed the presence of antibiotic residues—especially tetracyclines—in fish species from Eleyele River, with *Parachanna obscura* and *Clarias gariepinus* showing levels far above international safety limit(0.1µg/g). *Oreochromis niloticus* showed no detectable contamination. The findings suggest environmental pollution from unregulated antibiotic use, posing risks to both ecosystem and public health.

Recommendation

Stricter regulation of antibiotic use in agriculture and aquaculture near Eleyele River is urgently needed to address the high antibiotic residues found in fish. Regular monitoring, public education on the risks of antibiotic misuse, and promotion of safer practices should be prioritized. Encouraging the consumption of less-contaminated species like *Oreochromis niloticus* and exploring alternatives such as vaccines and probiotics can help reduce environmental and health risks.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgment

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