# Detection of Antibiotics Residues in two species of fish in Shatt Al-Arab River, Southern Iraq

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## Abstract

The presence of antibiotics in the aquatic environment is a major concern because of the effect of antibiotics on water quality, aquatic organisms and human health. The current study aims to detect antibiotics (Amoxicillin, Ciprofloxacin, and Levofloxacin) seasonally in the muscles and liver of two species of fish (*Oreochromis niloticus*) and (*Planiliza abu*) during the period from November 2020 to August 2021 in two selected stations in Shatt Al-Arab, Basrah, Iraq. The samples were analyzed using high performance liquid chromatography (HPLC), High concentrations of antibiotics were recorded in the muscles and liver of fish during the study period. The highest concentration of antibiotics Amoxicillin (AMO) was recorded in the muscles of *O. niloticus* during the spring season (8.7 mg. kg-1). antibiotics concentrations Ciprofloxacin (CIP) were also high in most seasons of the study. The presence of antibiotics in the aquatic environment in such high concentrations is a source of great concern. The study recommends conducting more studies to measure antibiotics contamination in other areas of the Shatt Al-Arab and the Iraqi marshes and effluent of hospitals.

**Keywords:** antibiotics, Aquatic environment, Fish, Pollution, Shatt Al-Arab.

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## Introduction

Antibiotics are substances that reduce or prevent the reproduction and growth of microorganisms that infect humans, including bacteria, fungi and microalgae (Cheng *et al*., 2017; Torres *et al*., 2017). The role of antibiotics was not limited to treating infectious diseases in humans, but also extended to animals of all kinds (Thakare *et al*., 2020). antibiotics are classified into three different classes: natural antibiotics, semi-synthetic antibiotics, and synthetic antibiotics. Natural antibiotics are produced by microorganisms (bacteria and fungi) such as penicillin and gentamicin to inhibit or kill other competing microorganisms, and semi-synthetic antibiotics are natural antibiotics are chemically modified by introducing an additive into the drug formulation, which improves their efficacy (more stable and less biodegradable), while synthetic antibiotics are produced entirely from chemicals based on the basic principle of natural antibiotics (Grenni *et al*., 2018). Pollution with antibiotics in the environment is due to many factors, not all of the antibiotics used in the treatment of humans and animals are absorbed into the body and are excreted with sewage into the aquatic environment. In addition, most of the remaining unused antibiotics from laboratories, pharmaceutical factories, residential and commercial areas and hospitals are disposed of in the water stream (Qiao *et al*., 2018; Ngigi *et al*. 2019).

There are no local studies on antibiotic contamination except for Mahmood *et al*. (2019) which examined the detection of antibiotics in drinking water treatment plants in Baghdad, Iraq. The study concluded a high concentration of antibiotics is present in the drinking water of both Al Wahda and Al Rasheed stations. There are many international studies that dealt with the topic of antibiotic pollution, including the study Anh *et al*. (2020), which examined how the occurrence, sources and potential environmental risks of antibiotic pollution in the surface waters of East and Southeast Asian countries, as antibiotics were detected everywhere. In the surface waters of these countries with concentrations ranging from less than 1 ng $L^{-1}$ to hundreds of μg $L^{-1}$. Lee *et al*. (2021) evaluated the environmental risks of the antibiotics Amoxicillin, Enrofloxacin, Neomycin on the aquatic environment, where the study conducted a series of toxicity tests for these antibiotics on algae and bacteria, the results indicated that Amoxicillin and Enrofloxacin are antibacterial that cause great concerns for the environment, and that more efforts, studies and investigations are needed to investigate the environmental consequences caused by both antibiotics. As for the antibiotic Neomycin, where the study confirmed that the environment in the surrounding waters must be monitored before it can properly describe its environmental risks.

Also studied by Bilal *et al*. (2020). The effects of antibiotics in the aquatic environment and their stability and harmful effects, as he indicated that the presence of antibiotics is a major concern because these micro pollutants cause a kind of resistance to types of bacteria that have serious health damage to aquaculture, humans and livestock. Also, Bojarski and Witeska (2020) revealed antibiotics in the aquatic environment and their toxic effects on fish, as he confirmed that chronic exposure to

antibiotics by fish can cause physiological disorders such as blood changes, oxidative stress and histopathological changes, immunosuppression, metabolic disorders and general anxiety, as well as this study showed that low concentrations of antibiotics can affect the reproductive process, and that low concentrations of antibiotics can also affect aquatic bacterial communities causing changes in organisms. Minutes that live symbiotically with fish. Li and Cui (2020) also evaluated the environmental behavior of antibiotics in estuarine environments based on single and multiple interactions, pointing out the need to study the fate of antibiotics in aquatic environments to reveal the contamination status. The current study aimed to detect antibiotics seasonally in two types of fish in two selected stations in Shatt Al-Arab.

## Materials and Methods

### Description of the study area

The Shatt al-Arab is one of the important rivers, the Shatt al-Arab consists of the confluence of the Tigris and Euphrates rivers at the city of Qurna, north of the city of Basrah, and then extends in the southeast direction for a distance of approximately 195 km to drain into the Arabian Gulf south of the town of Faw. The river's width of the ranges from 400 m in the city of Basrah to About 1500 m near Ras Al-Bishah after its confluence with the Karun River, and its depth ranges between 8-15 m, and the depths may reach more than that in some areas. (Almahmood *et al*., 2011). The southern part of Shatt Al-Arab River suffers from tidal phenomenon as a result of the entry of the Gulf waters to it, so the quality of the downstream water becomes mixed between marine and fresh (Abdullah *et al*., 2015).

In this study, two stations were selected from Shatt Al-Arab to detect antibiotics (Figure 1). The first station is located in the center of Basrah city near Al-Sadr Teaching Hospital 30°30'33"N 47°51' 03"E, It is located near a dock for commercial ships, and the movement of recreational boats and fishing boats is active, in addition to the presence of many tourist restaurants that throw their waste into the river, In addition to its proximity to Al-Sadr Teaching Hospital, where it is considered a source of great pollution to the river. The second station is located near the Salhiya River, within latitude and longitude 30°30'24"N 47°51'27"E. It is about 2 km away from the first station. The movement of recreational boats, transport and fishing boats is also active, and the area is affected by the water coming from the Salhia River, which contributes to increasing the pollution of the area.

### Sample collection

Fish samples were collected from the two selected study stations seasonally over a full year, from November 2020 to August 2021.

Two fishing methods were used to collect fish samples, which are the drift gill net, which is 120 m long and its holes are 15 × 15 mm, and the cast net has a diameter of 9 m and the size of its holes 15 × 15 mm. The caught fish are kept in a cork container containing crushed ice until returned to the laboratory. The weights of fish caught during the study ranged from 15 - 77g.

## Detection of antibiotics

## Preparation of standard solutions

Standard solutions were prepared at a concentration of 20 mg. $L^{-1}$ of Amoxicillin and 10 mg. $L^{-1}$ of Ciprofloxacin, Levofloxacin by dissolving the pure substances in D.D.W. (Hamscher *et al.*, 2002; Gros *et al.*, 2006), Standard solutions were injected into the HPLC device in order to draw the standard curve, which is used to compare with the curve of the sample to estimate the amount of antibiotics it contains.

## Solid-Phase Extraction (SPE)

In order to perform a quantitative analysis of each of Amoxicillin, Ciprofloxacin, and Levofloxacin in the sample (muscles & liver), 10gm of the sample was taken and placed in a volumetric vial with a capacity of 250 ml and 100 ml (methanol: distilled water) (1:1) was added to it and mixed for one hour on a magnetic stirrer. Then it was placed in a sonic boom device for 30 minutes, after which the sample was filtered through a 0.45 µm filter. The final volume was completed to 250 ml with distilled water. The sample was stored in the refrigerator for analysis by HPLC.

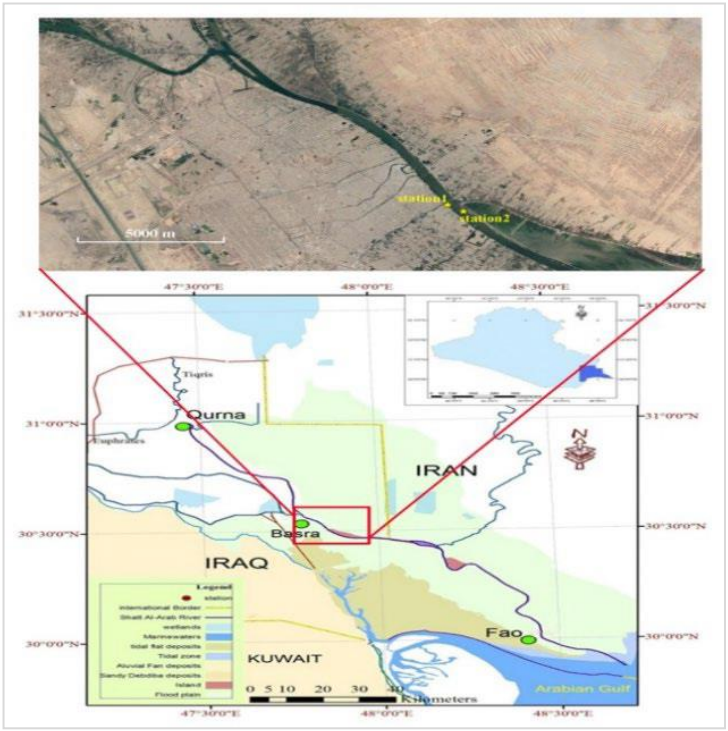


Figure 1: A map showing the two sampling stations.

## Analytical methods

### 1- Amoxicillin

The examination was conducted in the laboratories of the Ministry of Science and Technology, Department of Environment and Water using a high-performance liquid chromato-graphy device (HPLC) model (SYKAMN) of German origin and according to the conditions mentioned in the source (P1500 pump, UV2000 detector, AS3000 automatic sampling device). (Unutkan *et al*., 2018) used the carrier phase consisting of (acetonitrile: methanol: phosphate buffer) according to the following ratios (10:30:60) (V/V/V), and a separation column (C18- ODS (25 CM X) was used. 4.6 mm) using an ultraviolet detector (UV - 230 nm) at a flow rate (1 ml min $^{-1}$).

### 2- Ciprofloxacin and levofloxacin

The examination was conducted in the laboratories of the Ministry of Science and Technology, Department of Environment and Water using a high-performance liquid chromate-graphy device (HPLC) model (SYKAMN) of German origin and according to the conditions mentioned in the source. (Naveed *et al*., 2014) used the carrier phase consisting of (methanol: distilled water) according to the following ratios (70:30) (V/V), and a separation column (C18 - ODS (25 cm x 4.6 mm) using a radiation detector was used. Ultraviolet (UV - 294 nm) at a flow rate (1 ml,min$^{-1}$).

### Statistical analysis

The statistical program Statistical Package for Social Science (SPSS) (V. 20) used to conduct the statistical analysis of some of the study results under the significance level of (0.05).

## Results and Discussion

The presence of antibiotics even at low levels in the aquatic environment is more than enough to be contaminated and lead to adverse effects on water quality and aquatic organisms. Two groups of antibiotics Fluoroquinolone (Ciprofloxacin, Levofloxacin) and B-lactam (Amoxicillin) were detected in this study.

### Antibiotics in fish

Fish were used as bio indicators to know the extent of the organism's response to environmental variables and its resistance to pollution, so it became one of the useful tools in environmental monitoring.

Figs. (2 and 3) show the seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin and Ciprofloxacin,) in the liver and muscles of Nile tilapia fish during the study period.

Figs. (4 and 5) show the seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin and Ciprofloxacin,) in the liver and muscles of *P. abu* fish during the study period.

This study is the first to measure antibiotics concentrations in fish, as antibiotics were detected quarterly in the muscles and liver of fish for a whole year.

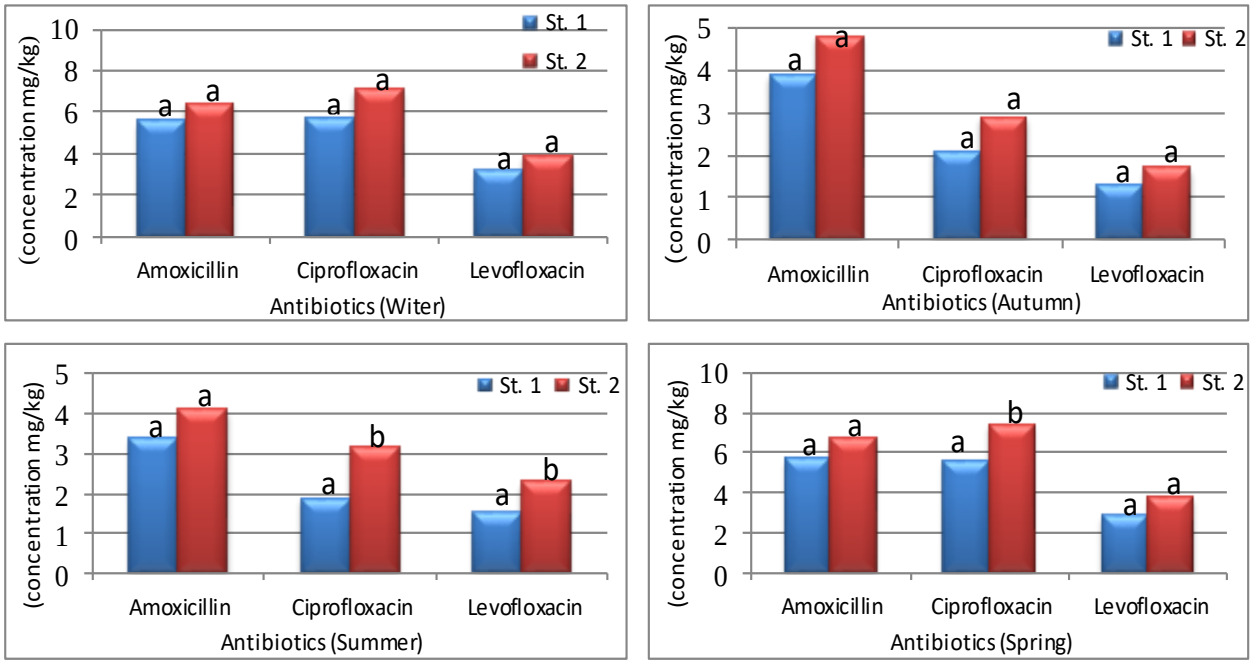


Figure 2: Seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin and Ciprofloxacin) in the muscles of *O. niloticus* fish during the study period, (different letters in the stations are significantly different (P<0.05).

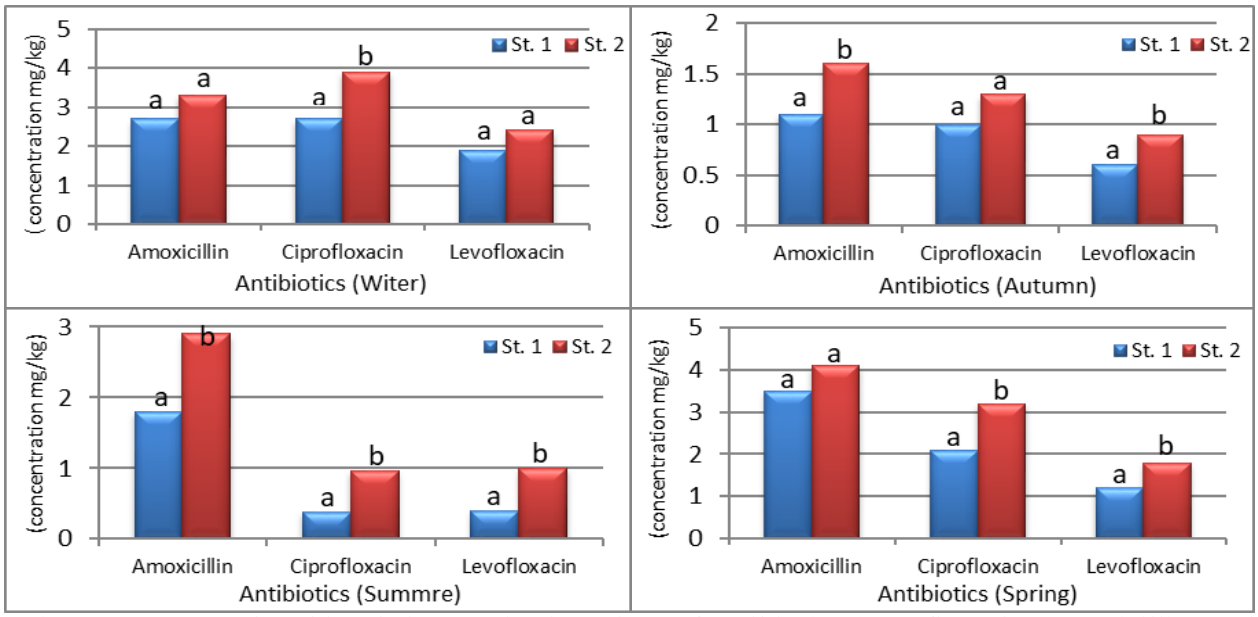


Figure 3: Seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, and Ciprofloxacin) in livers of *O. niloticus* fish during the study period, (different letters in the stations are significantly different (P<0.05).

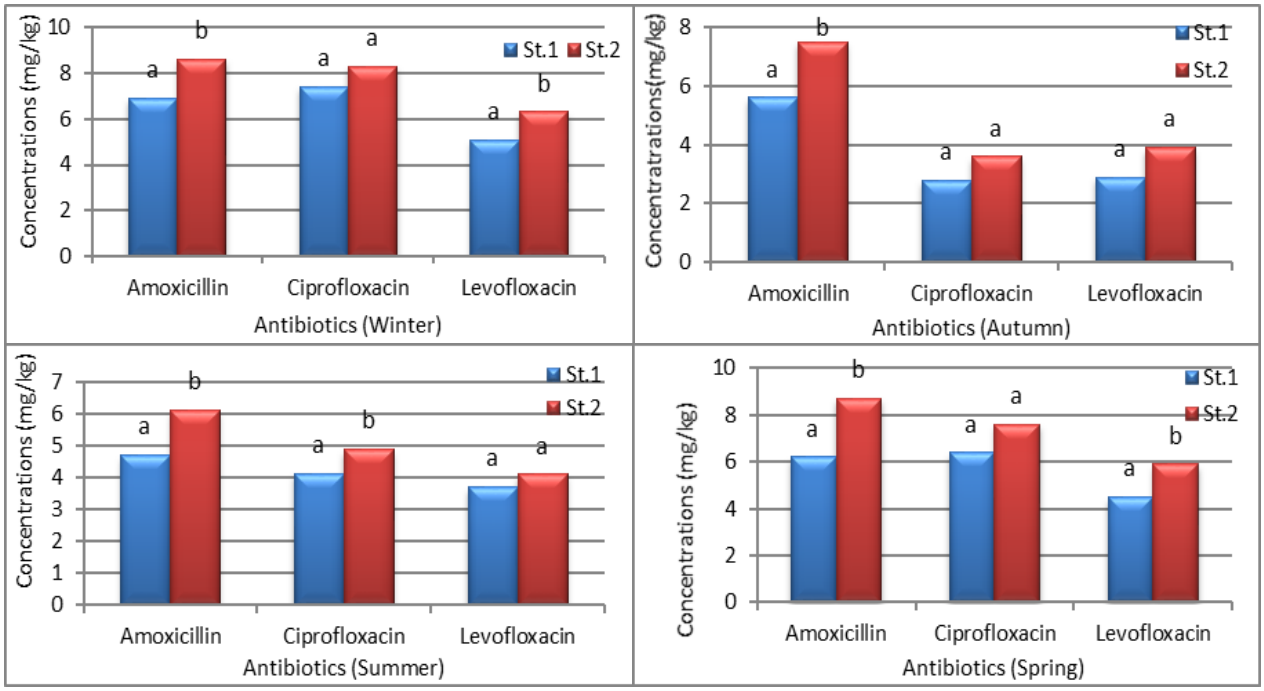


Figure 4: Seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin and Ciprofloxacin) in the muscles of *P. abu* fish during the study period, (different letters in the stations are significantly different (P<0.05).

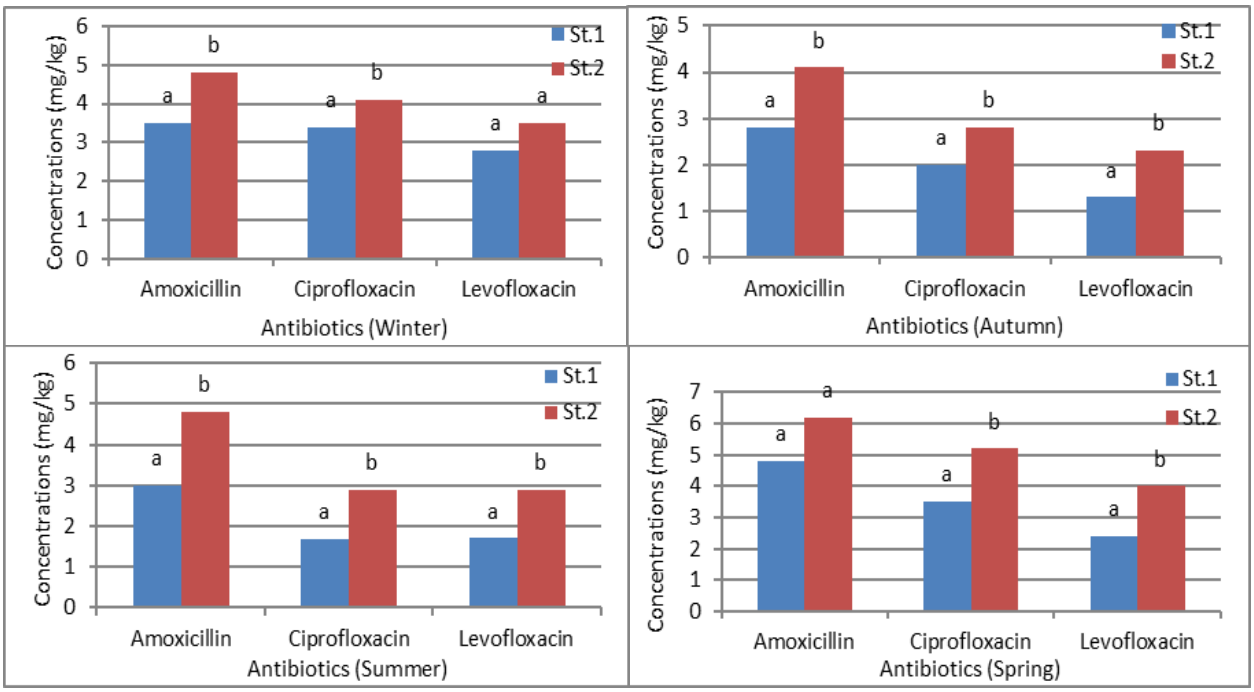


Figure 5: Seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, and Ciprofloxacin) in livers of *P. abu* fish during the study period, (different letters in the stations are significantly different (P<0.05).

This study is the first in Iraq that revealed antibiotics residues in fish, where antibiotics were detected seasonally in the muscles and liver of a mentioned fish for a whole year, The results of the current study show a significant variation in antibiotics values in the muscles and liver of fish between height and decline, The reason for this may be due to the high regulating ability of fish through the physical and chemical composition of their tissues and their ability to release pollutants when they reach the critical limit (Reddy *et al.*, 2007). The results also showed that most of the concentrations of antibiotics recorded in the current study are very high and are considered of a high risk to the aquatic environment, and may have direct health risks to humans in different age groups, especially the antibiotics (CIP), which is considered toxic to children from the age of one day to three months (Cui *et al.*, 2018).

The results of the study also showed that the accumulation of antibiotics was higher in the fish muscles than in the liver, and this may indicate the stability of this type of pollution inside the fish body, considering that the muscles are the last part in which the absorption or accumulation of pollutants occurs, since the muscles are inactive tissues. Studies indicate that chronic exposure of fish to environmentally realistic low concentrations of antibiotics may lead to physiological disturbances such as hematological changes, oxidative stress, pathological tissue lesions, immunosuppression, metabolic disturbances, genotoxic damage, general stress response, and reproductive impairment (Unutkan *et al.*, 2018).

## Conclusions

1. The study recorded a significant increase in the level of contamination with antibiotics, and this may be due to the absence of environmental control and the release of all kinds of pollutants and sewage water in particular.
2. The second station recorded high concentrations of antibiotics compared to the first station.
3. The results of the study showed that the concentration of the antibiotics Ciprofloxacin was high during the study period.
4. Concentrations of antibiotics were higher during the cold seasons (winter and spring) than during the summer.
5. The concentration of antibiotics was higher in Nile tilapia compared to cod fish during the study period.
6. The concentration of antibiotics was higher in fish muscles than in liver during the study period.

## Acknowledgments

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## Ethical approval

All ethical guidelines related to animal care issued by national and international organizations were implemented in this report.

## Conflicts of Interest

The authors declare no conflicts of interest.

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# الكشف عن بقايا المضادات الحيوية في نوعين من الأسماك في نهر شط العرب جنوب العراق

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**المستخلص**

يعد وجود المضادات الحيوية في البيئة المائية مصدر قلق كبير بسبب تأثير المضادات الحيوية على جودة المياه والكائنات المائية وصحة الإنسان. تهدف الدراسة الحالية إلى الكشف عن المضادات الحيوية (Amoxicillin, Ciprofloxacin, Levofloxacin) فصلياً في عضلات وكبد نوعين من الأسماك (*Oreochromis niloticus*) و (*Planiliza abu*)خلال الفترة من تشرين الثاني 2020 إلى آب 2021 في محطتين مختارتين في شط العرب، البصرة، العراق. تم تحليل العينات باستخدام التحليل الكروماتوغرافي السائل عالي الأداء (HPLC)، وتم تسجيل تراكيز عالية من المضادات الحيوية في عضلات وكبد الأسماك خلال فترة الدراسة. تم تسجيل أعلى تركيز للمضاد الحيوي Amoxicillin (AMO) في عضلات البلطي النيلي خلال فصل الربيع (8.7 ملغم/لتر). وكانت تركيزات المضادات الحيوية Ciprofloxacin (CIP) مرتفعة أيضًا في معظم فصول الدراسة. إن وجود مضادات الحيوية في البيئة المائية بمثل هذه التراكيز العالية يشكل مصدر قلق كبير. وتوصي الدراسة بإجراء المزيد من الدراسات لقياس التلوث بالمضادات الحيوية في مناطق أخرى من شط العرب والأهوار العراقية ومتدفقات الخارجة من المستشفيات.

**الكلمات المفتاحية:** المضادات الحيوية، البيئة المائية، الأسماك، التلوث، شط العرب.

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