



Detection of Antibacterial Residues in Nile Tilapia *Oreochromis niloticus* (L.) in the Shatt Al-Arab river, Southern Iraq

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Abstract: The presence of Antibacterial in the aquatic environment is a major concern because of the effect of Antibacterial on water quality, aquatic organisms and human health. The current study aims to detect Antibacterial (Amoxicillin, Ciprofloxacin, and Levofloxacin) seasonally in water, sediments, muscles and liver of Nile tilapia fish 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 the antibiotic Ciprofloxacin (CIP) were recorded in each of the water, sediment and fish muscle samples during the spring season at the second station, reaching the 24.9 mg l⁻¹, 24.5 mg. kg⁻¹, 7.4 mg. kg⁻¹, respectively, while the high concentration of Amoxicillin (AMO) Antibacterial was 4.1 mg.kg⁻¹ in liver of fish in spring season in the second station. The presence of Antibacterial in the aquatic environment in such high concentrations is a source of great concern. The study recommends conducting more studies to measure Antibacterial contamination in other areas of the Shatt al-Arab and the Iraqi marshes.

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

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). Antibacterial 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 Antibacterial 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 & 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 stress, 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 lives symbiotically with fish. Li & 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 in order to reveal the contamination status. The current study aimed to detect antibiotics seasonally in water, sediments and fish of Nile tilapia in two selected stations in Shatt Al-Arab.

Materials & 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 city of Faw. The width of the river 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 that 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 in water, sediments and Nile tilapia fish (Fig. 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 Sallhia River, which contributes to increasing the pollution of the area.

Sample collection

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

Water samples

Water samples were collected a seasonally from the two stations using litre glass bottles that were filled to their full capacity and all

were kept in a cool box containing ice cubes until reaching the laboratory.

Sediments samples

Sediment samples were collected from the the surface layer at a distance of 1-2 m from the cliff. Samples were taken from areas covered with water continuously and kept in plastic bags until reaching the laboratory.

Fish samples

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 on crushed ice until return to the laboratory. The weights of fish caught during the study ranged from 15-77 g.

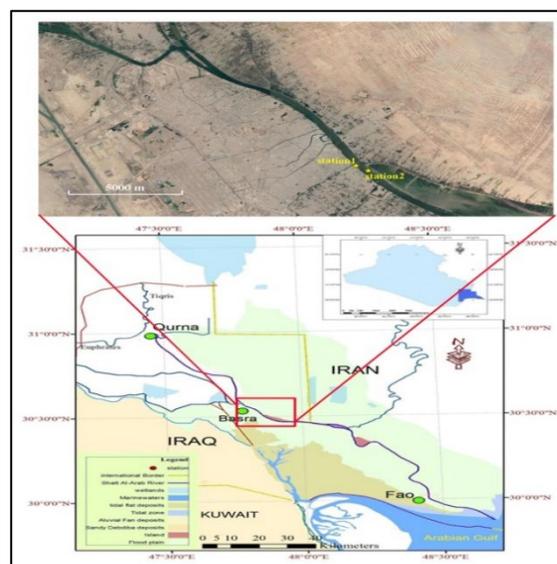


Fig. (1): A map showing the two sampling stations.

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 and 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.

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 chromatography 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: phosphite 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 chromatography 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 & Discussion

Antibiotics in water

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 (Table 1).

This is the first study in Iraq that indicates the presence of antibiotics in the aquatic environment (Shatt Al-Arab). Fig. (2) shows the seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, and Ciprofloxacin) in the water during the study period. The results showed that Fluoroquinolones (Ciprofloxacin) were the most frequently detected antibiotics in the water samples, where high concentrations of CIP antibiotic were recorded in the second station throughout the study period, especially during the winter season.

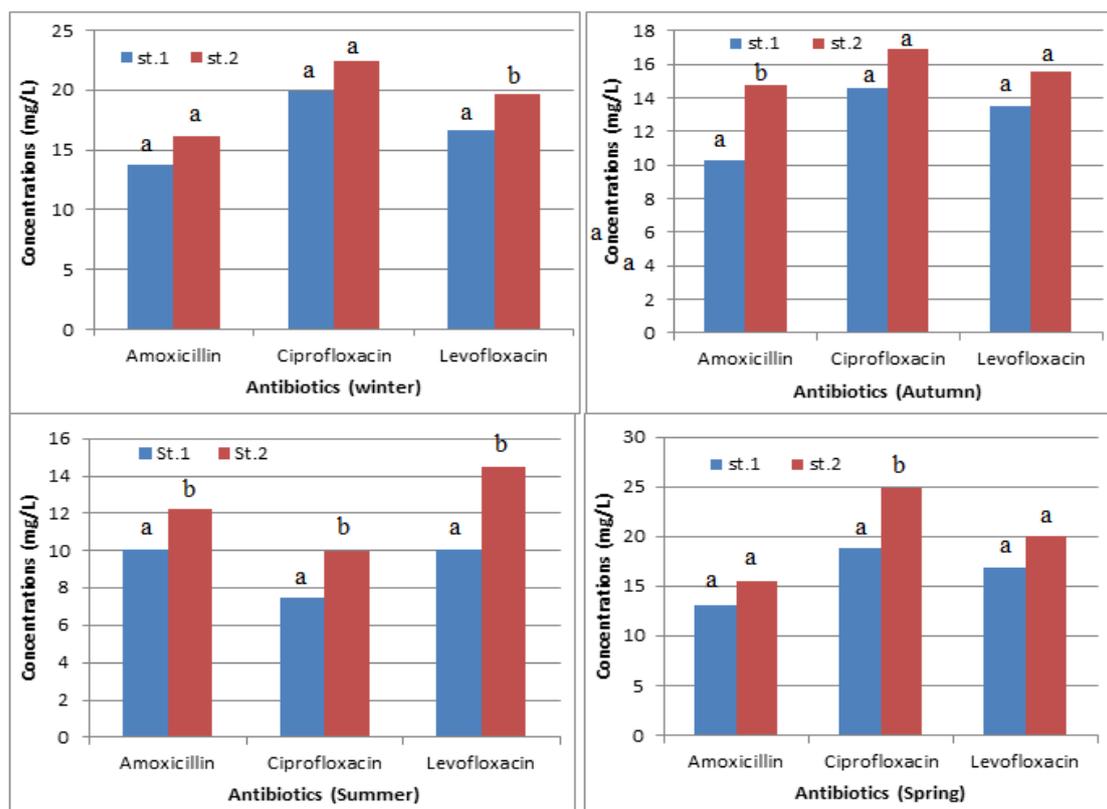
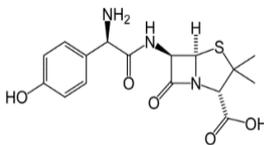
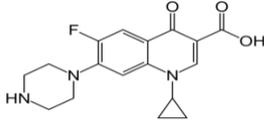
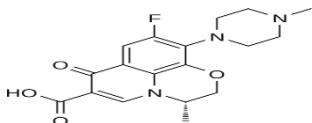


Fig. (2): Seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, and Ciprofloxacin,) in water during the study period, (different letters in the stations are significantly different ($P < 0.05$)).

Table (1): Shows the aggregates, the chemical structure and the molecular formula of the antibiotics (Amoxicillin, Ciprofloxacin, Levofloxacin) to be detected using the HPLC device. (Mahmood *et al.*, 2019)

Antibiotics	Antibiotic class	Formula	Chemical structures	Mol. Wt. (g. mol ⁻¹)
Amoxicillin	B-lactam	C ₁₆ H ₁₉ N ₃ O ₅ S		365.40
Ciprofloxacin	Fluoroquinolone	C ₁₇ H ₁₈ FN ₃ O ₃		331.34
Levofloxacin	Fluoroquinolone	C ₁₈ H ₂₀ FN ₃ O ₄		361.37

This may indicate the effect of sewage that is dumped without treatment into the Shatt Al-Arab waters because the antibiotic CIP is only used for human treatment, in addition to the possibility of contamination from an animal source because the antibiotic enrofloxacin is only used for animal treatments where it can be metabolized under certain conditions to Ciprofloxacin, which led to increase its concentration in water. As explained by Blackwell *et al.* (2005) that the high concentrations of antibiotics, especially ciprofloxacin, are not only due to their continued release into the environment, but also to high internal stability and resistance to degradation, which leads to their wider spread and their survival in the environment for a longer period and in higher concentrations. As for the lowest values, they were also recorded for CIP antibiotics in the summer, this decrease does not mean its decomposition and

its fading from the water because it is a difficult antibiotic to biodegrade, but rather it is due to its high adsorption efficiency on sediments (Li & Zhang, 2010).

Antibiotics in sediment

Sediments are a complex mixture of a number of soil forms that may include clay, silica, organic matter, carbonate, and a large number of bacteria (Al-Khazrajy & Boxall, 2016). Fig. (3) shows the seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, and Ciprofloxacin,) in the sediment during the study period, The lowest values were recorded during the fall season for the antibiotic (LEV), which amounted to 9.8 mg. kg⁻¹ and 11.4 mg .kg⁻¹ for the first and second stations, respectively, while the highest values were recorded in the winter for CIP, reaching 18.9 mg. kg⁻¹ in the first station and in the spring for CIP 24.5 mg. kg⁻¹ in the second station.

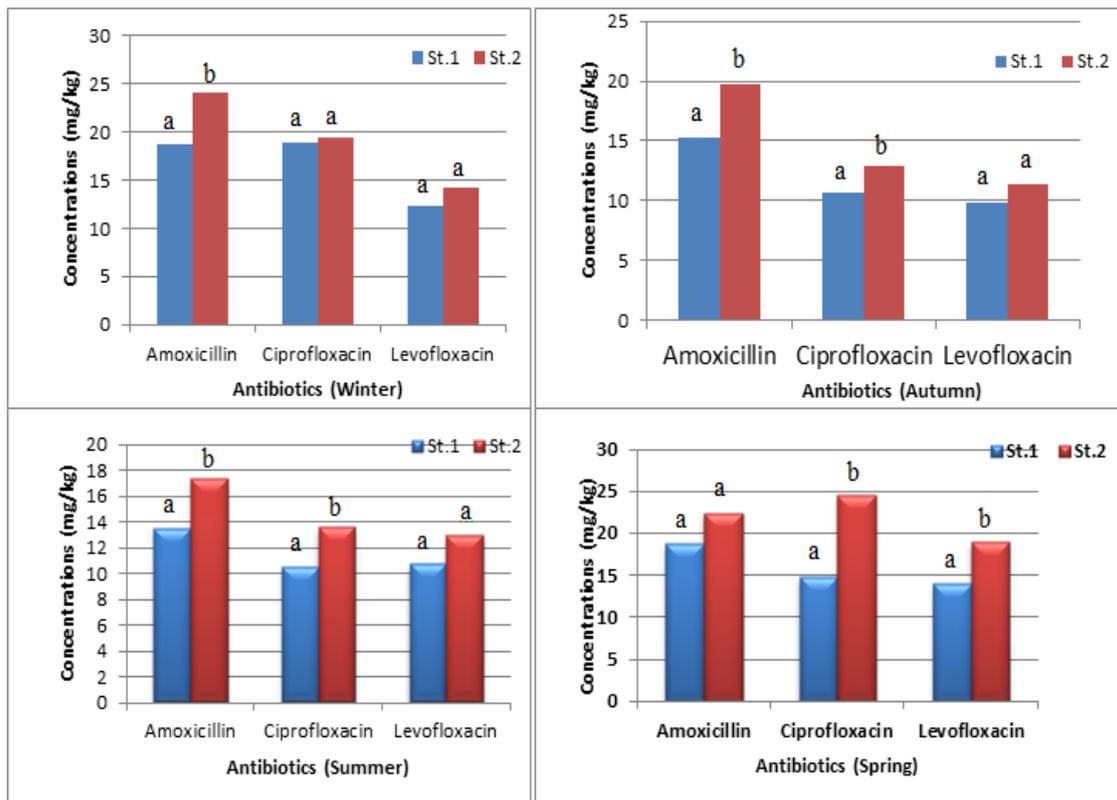


Fig. (3) Seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, Ciprofloxacin,) in sediments during the study period, (different letters in the stations are significantly different (P<0.05)).

The current study recorded high concentrations of antibiotics in sediments compared to those measured in water and fish samples, and this may be due to the fact that the sediments act as a reservoir for pollutants, or the reason for this may be due to the tendency of most suspended particles to bind and form more complex particles that are deposited after that in sediments gradually, This was confirmed by Al-Khafaji *et al.* (2011), who stated that sediments are the final sink for many suspended materials in the water column, whose ultimate fate is sediments.

Antibiotics in Nile tilapia

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. (4 and 5) 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, This study is the first to measure antibiotic concentrations in fish, as antibiotics were detected quarterly in the muscles and liver of Nile tilapia fish for a whole year.

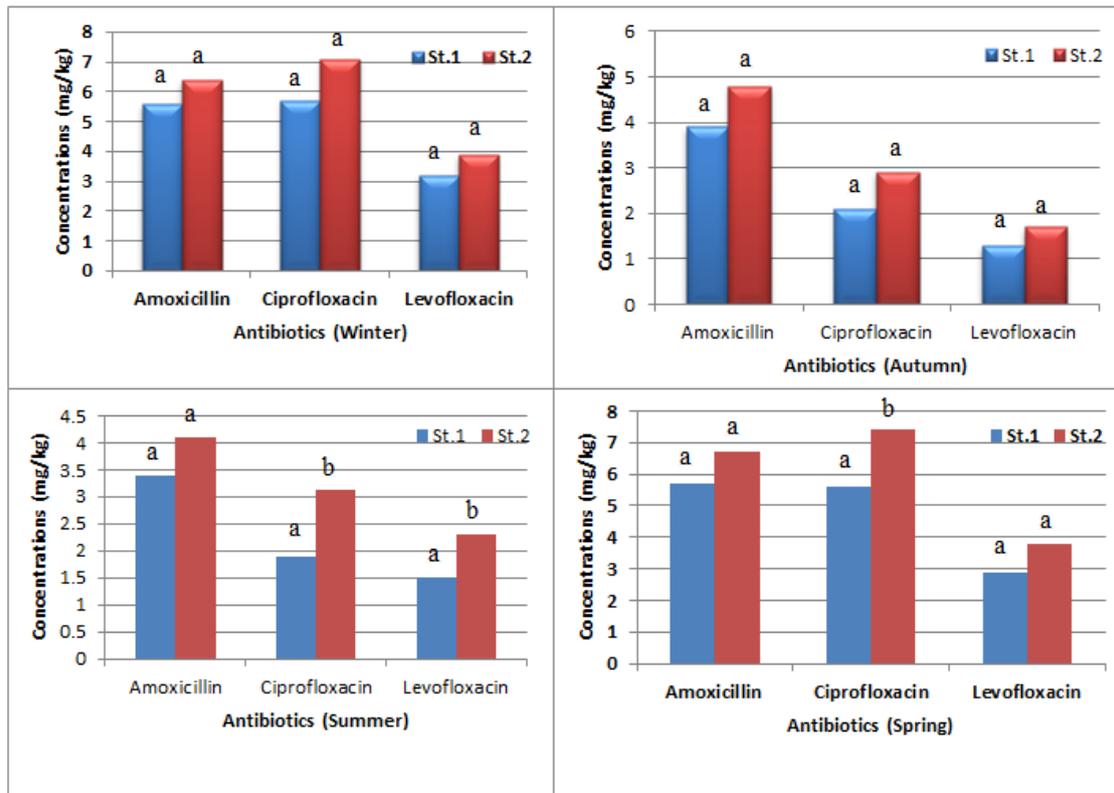


Fig. (4): Seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin and Ciprofloxacin) in the muscles of Nile tilapia fish during the study period, (different letters in the stations are significantly different (P<0.05)).

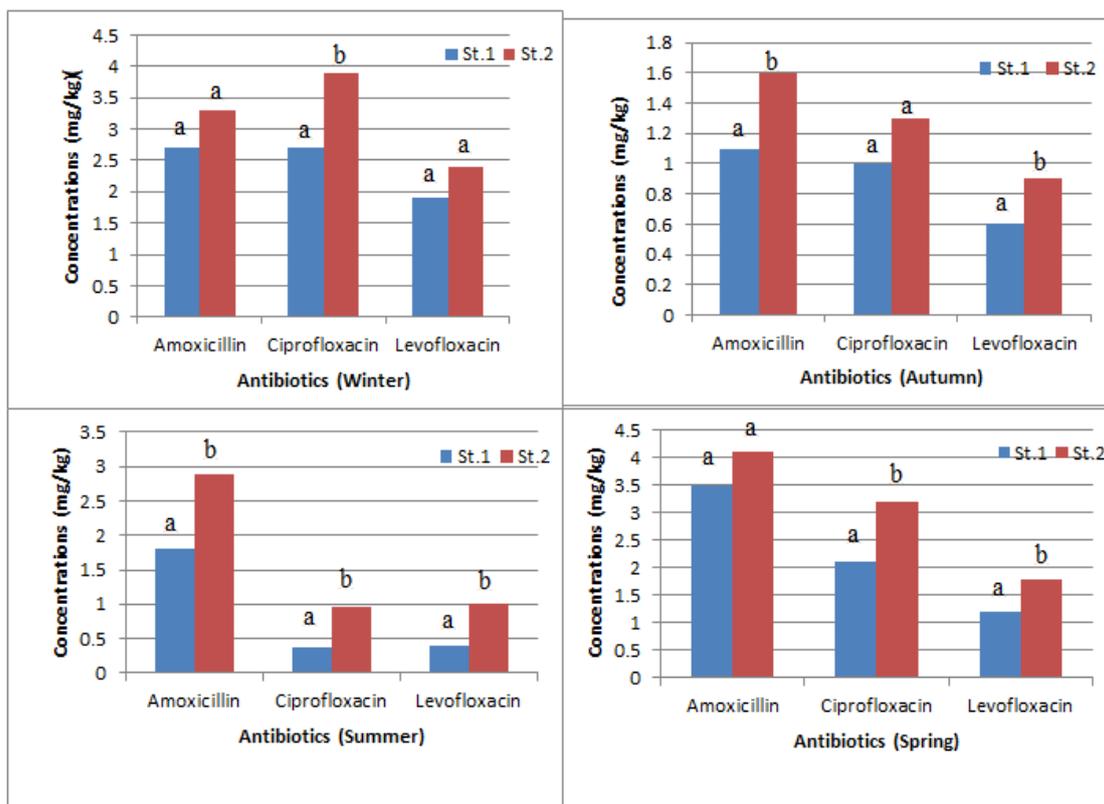


Fig. (5): Seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, and Ciprofloxacin) in livers of Nile tilapia fish during the study period.

The results of the current study showed a large variance in the values of antibiotics in the muscles and liver of Nile tilapia fish between high and low, and this may be due to the high regulating ability of fish through the physical and chemical composition of their tissues and their ability to excrete pollutants when they reach the critical limit (Reddy *et al.*, 2007).

The results 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 environment and aquatic organisms, and may have direct health risks to humans in different age groups, especially the antibiotic CIP, which is considered toxic to children from the age of one 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 extent

of the persistence 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 (Ahmed *et al.*, 2022).

Conclusion

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 antibiotic Ciprofloxacin was high during the study period.

4. Concentrations of antibiotics were higher during the cold seasons (winter and spring) than during the summer.

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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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Contributions of Authors

M.H.A.: Sample collection, Laboratory methodology, and writing the manuscript.

K.S.A.: Suggest a title of the research, graphs, statistical analysis, and editing revision.

A.K. R.: Suggest a title of the research, and sample collection.

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الكشف عن بقايا المضادات الحيوية في اسماك البلطي النيلي (*Oreochromis niloticus* (L.)) في شط العرب، جنوب العراق

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المستخلص: أن وجود المضادات الحيوية في البيئة المائية يشكل مخاوف كبيرة بسبب تأثيرها على نوعية المياه والأحياء المائية وصحة الإنسان. تهدف الدراسة الحالية الكشف عن المضادات الحيوية (Amoxicillin و Ciprofloxacin و Levofloxacin) فصلياً في المياه والرواسب وعضلات وكبد أسماك البلطي النيلي خلال الفترة من تشرين الثاني 2020 ولغاية آب 2021 في محطتين مختارة من شط العرب، البصرة، العراق. تم تحليل العينات باستخدام كروماتوغرافيا السائل عالي الأداء (HPLC)، أحتل المضاد الحيوي CIP التركيز الأعلى في عينات المياه والرواسب وعضلات اسماك البلطي النيلي 24.9 ملغم. لتر⁻¹، 24.5 ملغم.كغم⁻¹، 7.4 ملغم. كغم⁻¹ على التوالي في فصل الربيع في المحطة الثانية، بينما كان تركيز المضاد الحيوي AMO عالي 4.1 ملغم كغم⁻¹ في كبد أسماك البلطي النيلي في فصل الربيع في المحطة الثانية. وجود المضادات الحيوية في البيئة المائية بهذه التراكيز العالية هو مصدر قلق كبير، توصي الدراسة بأجراء المزيد من الدراسات لقياس التلوث بالمضادات الحيوية في مناطق أخرى من شط العرب وأهوار العراق.

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