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Original Article

Effect of antibiotic residues on some health parameters of Nile tilapia, *Oreochromis niloticus* L. collected from Shatt Al-Arab

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Abstract: The presence of antibiotics in the aquatic environment poses great concerns due to their impact on water quality, aquatic organisms, and human health. The current study aims to detect the antibiotic residues (Amoxicillin (AMO), Ciprofloxacin (CIP), and Levofloxacin (LEV)) seasonally in the muscles and liver of Nile tilapia, *Oreochromis niloticus*, collected from Shatt Al-Arab and to know their effect on some health aspects of fish during November 2020 to August 2021 by selecting two stations in the river. The samples were analyzed using high-performance liquid chromatography (HPLC) for measuring antibiotics. The study revealed high concentrations of antibiotics in the muscles and liver of fish, and the concentrations were higher in the second station. CIP had the highest concentration (7.4 mg/kg) in muscles in the spring, and the AMO showed the highest concentration (4.1 mg/kg) in the liver during the spring. The study also showed that the accumulation of antibiotics in the liver and muscles of fish had negative effects on the health standards of fish. The presence of antibiotic residues in fish samples in high concentrations is a source of great concern as it is a major source of human food.

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Introduction

In recent years, antibiotics have been recognized as serious and active environmental pollutants due to their presence everywhere in high concentrations in surface waters, ground waters, soils, sediments, and animals in almost all parts of the world (Kovalakova et al., 2020; Lu et al., 2020). Pollution with antibiotics is attributed to many factors, including the release of antibiotics that are not absorbed by humans and animals into the water bodies. In addition, most of the remaining unused antibiotics from laboratories, pharmaceutical factories, residential and commercial areas, and hospitals are disposed of in the water systems (Qiao et al., 2018; Ngigi et al., 2020)

There are no local studies on antibiotic contamination in fish in Iraq, and there are few international studies that dealt with antibiotic contamination in fish, including the study of Fang et al. (2021) which dealt with the accumulation of antibiotics in *Oreochromis niloticus*. Their results showed that the accumulation of antibiotics in the muscles of the fish was positively related to its dose in the diet. This study also showed that the antibiotic SUL led to the inhibition of growth in Nile tilapia fish, emphasizing the need for rational and regulated use of antibiotics in aquaculture. Han et al. (2021) studied the spatial distribution and risk assessment of 14 antibiotics in typical mariculture farms surrounding the Bohai Sea in China and antibiotics were detected in seawater, sediments, some marine fish, mollusks, and sea cucumbers. Li et al. (2021) detected 12 antibiotics were detected in the muscle tissue of cultured aquatic organisms and in water and sediments in eastern China. This study also showed that the potential risks from ingesting these aquatic organisms are few and limited, except for the antibiotics belonging to the group of fluoroquinolones.

Bojarski and Witeska (2020) showed the presence

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of antibiotics in the aquatic environment and their toxic effects on fish species revealing chronic exposure cause physiological disturbances such as hematological changes, oxidative stress, histopathological changes, weak immunity, metabolic disorders, and general stress. Also, this study showed that low concentrations of antibiotics can affect the reproductive process. Low concentrations of antibiotics can also affect aquatic bacterial communities, causing changes in the microorganisms that live symbiotically with fish. Kondera et al. (2020) studied the effect of adding Oxytetracycline and Gentamicin in the diets on the hematological parameters of juvenile *Cyprinus carpio*. Their results showed no significant hematological or hepatotoxicity of therapeutic doses of OTC and GEN on juveniles, and this study also confirmed no significant changes in the values of hematological and biochemical parameters after taking OTC and GEN antibiotics. Nibamureke et al. (2019) studied the effect of the antibiotic Nevirapine on the liver of *O. mossambicus* in African surface waters under controlled conditions for 30 days concluding that NVP causes histological changes to liver cells and causes fibrosis around some veins and bile ducts and confirmed that long-term exposure to the antibiotic NVP causes negative effects on fish health. The current study aims to identify the effect of antibiotic residues accumulated in the liver and muscles of Nile tilapia on some health parameters collected from Shatt Al-Arab.

Materials and methods

Sampling: The Shatt al-Arab is the confluence of the Tigris and Euphrates rivers at the city of Qurna, north of Basrah, and then extends in the southeast direction for a distance of approximately 195 km to drain into the Persian Gulf. The width of the river ranges from 400 m in the Basra to about 1500 m near Ras Al-Bishah after its confluence with the Karun River. Its depth ranges from 8-15 m (AL-Mahmood et al., 2011). The southern part of the Shatt Al-Arab River suffers from tidal phenomenon as a result of the entry of the marine waters, so the quality of the downstream water becomes mixed with marine water (Abdullah et al., 2015).

In this study, two stations were selected from Shatt Al-Arab to detect antibiotics in water, sediments, and fish. The first station is located in the center of Basra city near Al-Sadr teaching hospital (30°30'33"N, 47°51'03"E) located near a dock for commercial ships, and the movement of recreational boats and fishing boats. In addition to the presence of many tourist restaurants that throw their waste into the river, it is considered a source of great pollution to the river. The second station was near the Salhiya River (30°30'24"N, 47°51'27"E). The movement of recreational boats, transport, and fishing boats is also active at this site, and the area is affected by the water coming from the Salhia River, which contributes to increasing pollution.

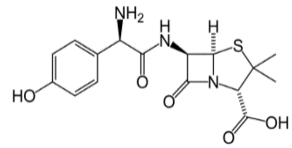
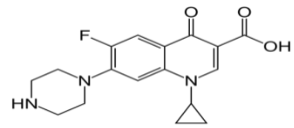
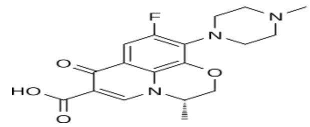
Fish samples were collected from the two stations seasonally from November 2020 to August 2021. Two fishing methods were used to collect fish samples, which are the gill net, 120 m long with a mesh size of 15 mm, and the cast net with a diameter of 9 m with a mesh size of 15 mm. The fish were kept in a cork container containing crushed ice until returning to the laboratory.

Detection of antibiotics

Preparation of standard solutions: Standard solutions were prepared at a concentration of 20 mg/L of Amoxicillin and 10 mg/L of Ciprofloxacin, and 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 to draw the standard curve, which is used to compare with the curve of the sample to estimate the value of antibiotics.

Solid-Phase Extraction (SPE): To measure the quantitative of Amoxicillin, Ciprofloxacin, and Levofloxacin in the sample, 10 g of the sample was taken and placed in a volumetric vial with a capacity of 250 ml and then 100 ml methanol: distilled water (1:1) was added and mixed for one hour on a magnetic stirrer. Then it was placed in a sonic boom device for 30 min, afterward which the sample was filtered through a 0.45 μm filter. The final volume

Table 1. The aggregates, the chemical structure and the molecular formula of the antibiotics (Amoxicillin, Ciprofloxacin, Levofloxacin) to be detected using the HPLC device.

Antibiotics	Antibiotic class	Formula	Chemical structures	Mol. Wt (g mol-)
Amoxicillin	B-lactam	C16H19N3O5S		365.40
Ciprofloxacin	Fluoroquinolone	C17H18FN3O3		331.34
Levofloxacin	Fluoroquinolone	C18H20FN3O4,		361.37

was completed to 250 ml with distilled water. The sample was stored in the refrigerator for analysis by HPLC.

Analytical methods

Amoxicillin: The examination was done using a high-performance liquid chromate-graphy device (HPLC) (SYKAMN, German) (P1500 pump, UV2000 detector, AS3000 automatic sampling device). The carrier phase consisting of acetonitrile: methanol: phosphite buffer was used according to the ratio of 10:30:60 (V/V/V), and a separation column (C18-ODS - 25 CM X) was applied. (4.6 mm) using an ultraviolet detector (UV-230 nm) at a flow rate of 1 ml/min (Unutkan et al., 2018).

Ciprofloxacin and Levofloxacin: The measurements were done in the laboratories of the Ministry of Science and Technology, Department of Environment and Water using a high-performance liquid chromate-graphy device (HPLC) (SYKAMN, German). The carrier phase consists of methanol: distilled water according to the ratios of 70: 30 (V/V), and a separation column (C18-ODS-25 cm x 4.6 mm) using a radiation detector was used with an ultraviolet (UV-294 nm) device at a flow rate of 1 ml/min (Naveed et al., 2014).

Hematological parameters: Blood samples were collected by drawing blood from the heart in a test tube containing 2.5% EDTA (Ethylenediaminetetra-acetic acid). The total protein of the blood plasma was measured by a ready-made laboratory kit (Randox, USA) using a spectrophotometer at a wavelength of 546 nm, according to the equation of total protein concentration (mg/ 100ml) = (sample reading/standard reading) x 6. The concentration of albumin in the blood plasma was measured by a ready-made kit (Randox, USA) using a spectrophotometer at a wavelength of 630 nm according to the equation of Albumin concentration (mg/ 100ml) = (sample reading/standard reading) x 4.5. Globulin concentration was measured by subtracting the albumin value from the total protein value for all samples (Wolf and Darlington, 1971) i.e. Globulin concentration (mg/ 100ml) = Total protein concentration - Albumin concentration. The Hemoglobin of the blood plasma was measured by a ready-made kit (Randox, USA) using a spectrophotometer at a wavelength of 570 nm, according to the equation of Hemoglobin concentration (mg/ 100ml) = (sample reading - standard reading).

Statistical analysis: The statistical program Statistical Package for Social Science (SPSS) was used to conduct the statistical analysis under the significance level of 0.05.

Results

In the current study, two groups of antibiotics, i.e. Fluoroquinolone (Levofloxacin (LEV), and

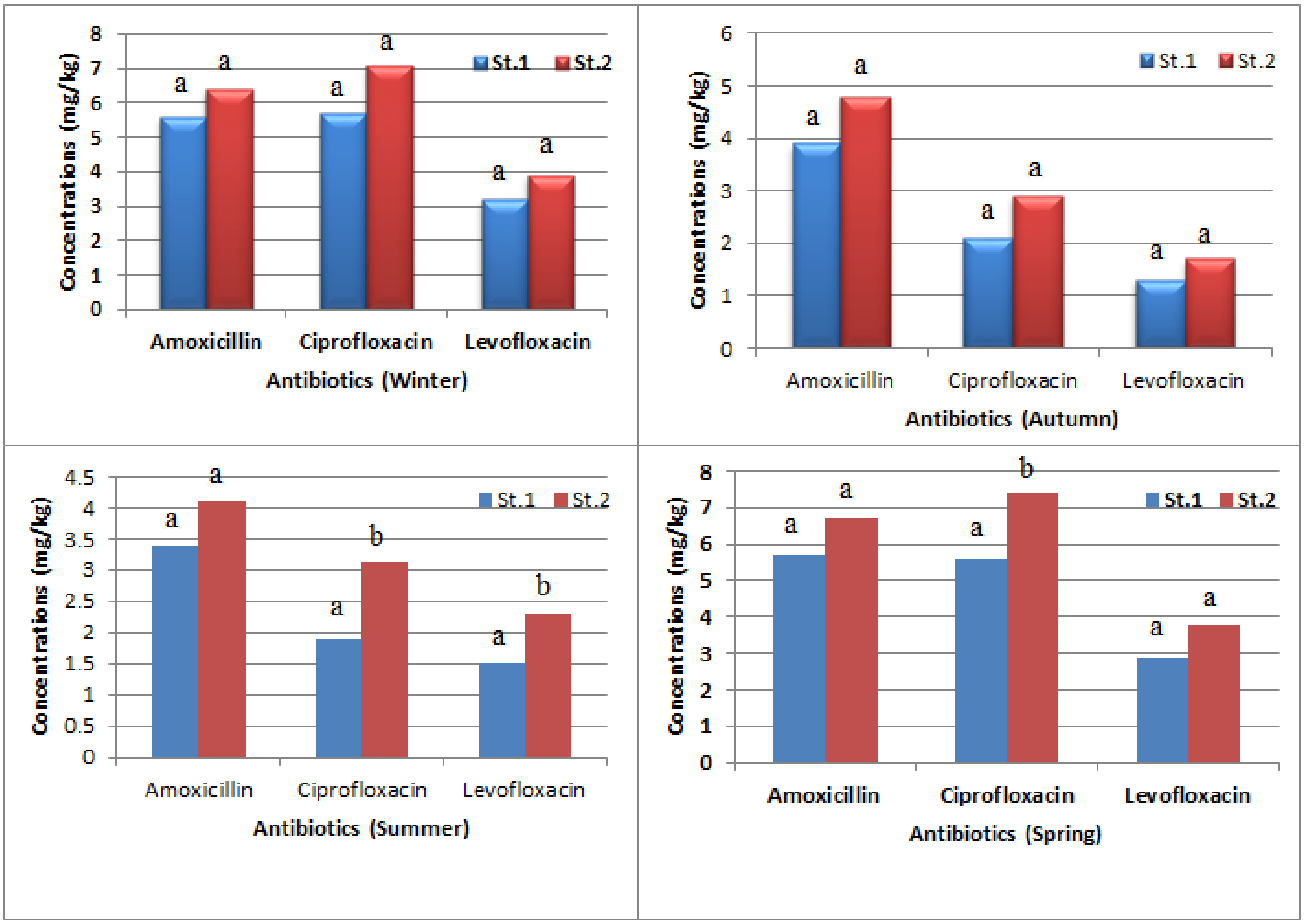


Figure 1. Seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, and Ciprofloxacin,) in the muscles of Nile tilapia fish during the study period (different letters in the stations are significantly different ($P<0.05$).

Amoxicillin (AMO)) and B-lactam (Ciprofloxacin, CIP) were detected (Table 1). The seasonal and local changes of the antibiotics (Levofloxacin, Amoxicillin, and Ciprofloxacin) in the muscles of Nile tilapia during the study period are shown in Figure 1. The lowest values were recorded at the first station during autumn for LEV (1.3 mg/kg), and the highest value recorded at the second station during spring was the CIP (7.4 mg/kg). Figure 2 shows the seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, and Ciprofloxacin,) in the liver of Nile tilapia during the study period. The lowest values were recorded at the first station during summer for CIP (0.38 mg/kg), and the highest values in the second station during spring for AMO (4.1 mg/kg).

Tables 2 show the seasonal and local changes in the total protein, albumin, globulin, and hemoglobin in Nile tilapia during the study period. The lowest values for total protein were recorded in the second station during the spring season (2.333 mg/100ml), and the highest values in the first station during autumn (3.88 mg/100 ml). The lowest values of albumin were recorded in the second station during summer (1.13 mg/100 ml), and the highest values in the first station during autumn (2.593 mg /100 ml).

The lowest values for globulin were recorded in the first station during the winter (0.953 mg/100 ml), and the highest values in the second station during the winter season (1.49 mg/100 ml). The lowest values of hemoglobin were found in the second station in spring (28.833%), and the highest values in the first station during the winter (53.1%).

Discussion

Fish were used as bioindicators to determine the extent of the organism's response to environmental variables and its resistance to pollution. The

Table 2. The concentrations of Albumin, Globulin, Total protein and Hemoglobin in the blood plasma of Nile tilapia fish in the two study stations.

Hematological parameters	Seasons (mean ± standard deviation)							
	Autumn		Winter		Spring		Summer	
	St.1	St.2	St.1	St.2	St.1	St.2	St.1	St.2
Total protein (mg/ 100 ml)	3.88 ± 0.144^{a}	3.566 ± 0.351^{a}	3.233 ± 0.236^{a}	2.57 ± 0.320^{a}	3.166 ± 0.208^{a}	2.333 ± 0.251^{a}	3.39 ± 0.441^{a}	3.1 ± 0.173^{b}
Albumin (mg/ 100 ml)	2.593 ± 0.061^{a}	1.52 ± 0.096^{b}	2.286 ± 0.155^{a}	1.32 ± 0.108^{b}	2.14 ± 0.069^{a}	1.213 ± 0.085^{b}	1.656 ± 0.228^{a}	1.13 ± 0.017^{b}
Globulin (mg/ 100 ml)	1.28 ± 0.105^{a}	1.366 ± 0.309^{b}	0.953 ± 0.055^{a}	1.49 ± 0.351^{b}	1.26 ± 0.208^{a}	1.143 ± 0.005^{b}	1.166 ± 0.06^{a}	1.153 ± 0.049^{a}
Hemoglobin (%)	42.833 ± 1.755^{a}	38.56 ± 1.115^{b}	53.1 ± 2.007^{a}	41.833 ± 3.329^{b}	41.333 ± 2.516^{a}	28.833 ± 1.04^{b}	38.733 ± 0.461^{a}	35.47 ± 2.2^{a}

Different letters in the same column (station 1 and station 2 for each adjective) mean that there are significant differences ($P<0.05$).

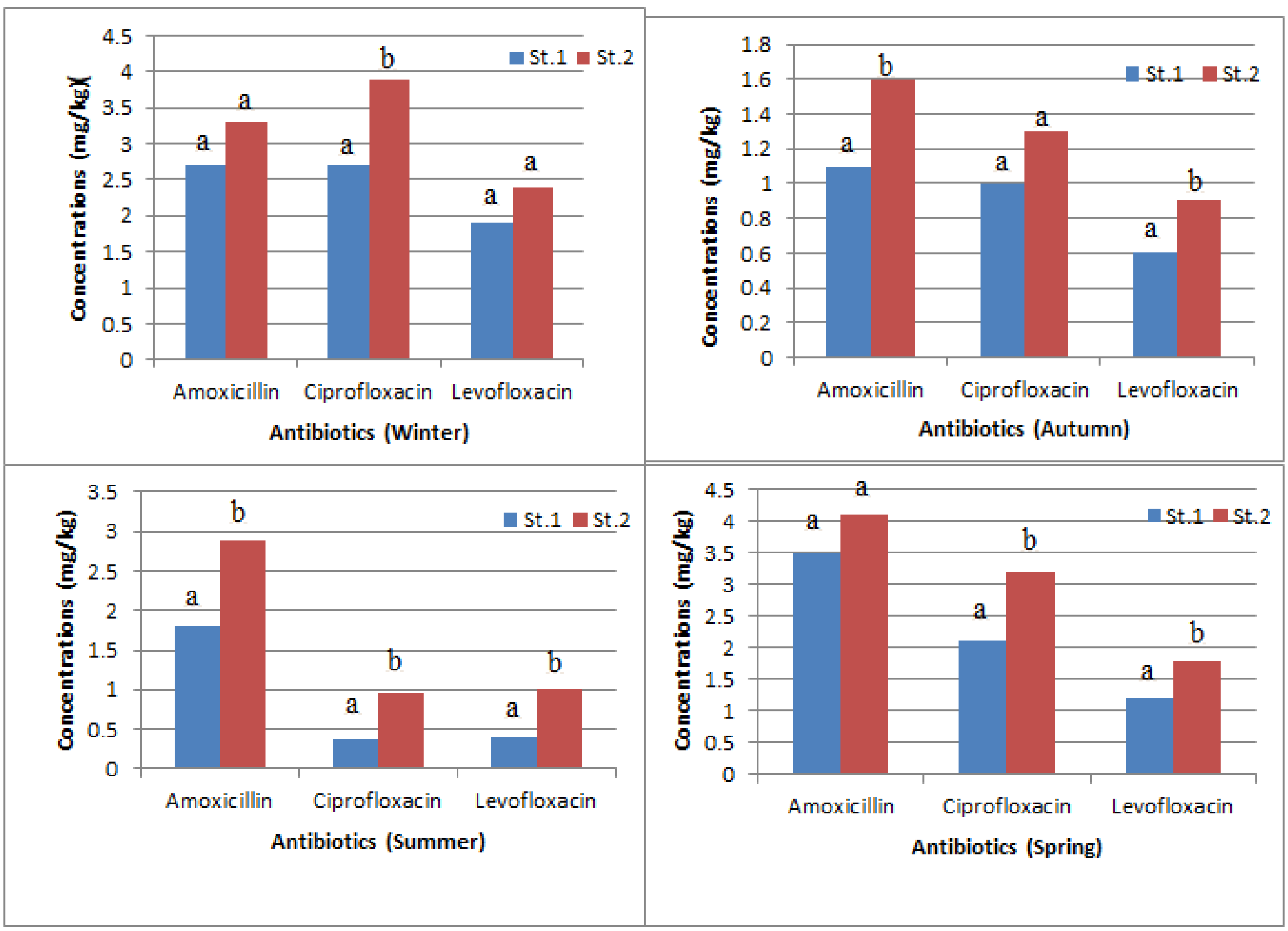


Figure 2. Seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, and Ciprofloxacin,) in livers of Nile tilapia fish during the study period (different letters in the stations are significantly different ($P<0.05$).

concentrations of antibiotics were higher in the second station than in the first station, and this may be due to its proximity to the Salhia River, which is polluted with sewage water. The results of the current study showed a large variation in the values of antibiotics in the muscles and liver of fish showing that most of the concentrations of antibiotics are very high and considered having high risk. These values have direct health risks to humans in different age groups, especially CIP, and its low concentrations are toxic to children of one to three months (Cui et al., 2018). Hu et al. (2010) and Kemper (2008) pointed out that the presence of antibiotics in the aquatic environment even at very low concentrations, causes chronic and hidden effects on fish behavior and the feminization of males in some fish species.

The results also showed that the accumulation of antibiotics was higher in the muscles of fish than in the liver, and this may the muscles are the last part in which the absorption or accumulation of pollutants occurs, since muscles are inactive tissues (Ben Salem et al., 2014). High concentrations of CIP were recorded in fish muscles, and this is probably due to its high stability and resistance to decomposition, which leads to its wider spread and its accumulation for a higher and longer period. The highest values of the AMO were recorded in the liver of fish, and this may be due to its high ability to accumulate and its high presence in the environment as a result of its wide use in the treatment of humans and animals. It is a broad-spectrum antibiotic used to treat many infections (Bielen et al., 2017). For LEV, it was recorded in low concentrations in the liver and muscles of fish, and this may be due to its rapid chemical breakdown.

Hematological parameters: Analysis of blood parameters is an important tool to assess the toxic effects of antibiotics on fish (Serezli et al., 2005; Burgos-Aceves et al., 2019; Bojarski and Witeska, 2020). Little is known about the effect of antibiotics on plasma or serum biochemical indicators in fish (Reda et al., 2013; Nakano et al., 2015; Hoseini and Yousefi, 2018). Changes in the blood of fish indicate the morphological and physiological changes that can occur due to the negative effects in the aquatic environment resulting from pollution (Javed and Usmani, 2014). Hoseinifar et al. (2011) and Carbone and Faggio (2016) mentioned that hematological parameters are useful tools for examining fish health and physiological responses and sensing ambient conditions affecting fish.

Changes in biochemical parameters (total protein, albumin, and globulin) can be attributed to changes in the liver. The results of the current study showed a slight variation in the total protein, albumin, and globulin between the two stations and fish. The highest values were recorded in the first station in tilapia. The rise in the level of these proteins may indicate an increase in their manufacture in the body and a decrease in the catabolism process, and the low level of both total protein and albumin in fish, it may be due to damage to blood vessels, and the exit of blood cells from the bloodstream into the tissues, causing hypoproteinemia (Bly et al., 1994). Kitancharoen and Hatai (1996) also mentioned that the low level of albumin in the blood plasma is due to the low efficiency of the liver in the formation of this protein and that its decrease is another reason for the decrease in the percentage of total protein because albumin constitutes 80% of it i.e. globulin-type proteins increase. In the current results, the level of globulin increased in the fish in the second station and decreased in the first station.

Hemoglobin is an important component of red blood cells and plays an important role in transporting oxygen in the blood of fish in general. Poor formation of red blood cells shows affecting their formation centers (kidney/spleen) and destroys red blood cells due to a change in the permeability of the cell membrane and an increase in mechanical fragility leads to a decrease in hemoglobin in the blood plasma of fish(Kondera et al., 2020). The results of the current study showed a variation in hemoglobin concentrations, and high concentrations of hemoglobin were recorded in tilapia fish during the winter. This may be due to the low concentrations of oxygen in the tissues as a result of exposure to pollution, and thus there is a need to produce red blood cells (Serezli et al., 2005). In addition, this increase may be due to the efficiency of the hormone Thyroxine, which is one of the hormones that regulate metabolism that this hormone causes an increase in metabolic reactions in tissues, which leads to an increase in the need for oxygen increasing hemoglobin (Thanikachalam et al., 2010).

Conclusions

The study recorded a significant increase in the level of antibiotic contamination in Nile tilapia. The second station recorded high concentrations of antibiotics compared to the first station. The results of the study also showed that the concentrations of the two antibiotics Amoxicillin and Ciprofloxacin were high during the study period with higher concentrations in spring.

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