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Detection Of Antibiotic Residues In Planiliza Abu H. In Shatt Al-Arab, Southern Of Iraq

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Abstract

Antibiotics represent one of the most important pollutants emerging in aquatic environments, which is a major concern for the environment and aquatic organisms. This study is considered the first in Iraq that dealt with the detection of antibiotic residues in the aquatic environment, as (Amoxicillin, Ciprofloxacin, Levofloxacin) were detected seasonally in water, sediments, and roughage Planiliza abu fish (muscle and liver) during the period from November 2020 to August 2021 in two stations. Selected from Shatt Al-Arab, Basra/Iraq. A set of environmental factors were measured during the study period, including water temperature (°C), pH and salinity (psu), where the results of the statistical analysis of environmental factors showed that there were no significant differences between the two study stations at a significant level (P>0.05). Antibiotics were detected in the laboratories of the Ministry of Science and Technology - Department of Environment and Water using a high-performance liquid chromatography (HPLC), The antibiotic (CIP) occupied the highest concentration in water and sediment samples 24.9 mg/ L, 24.5 mg/kg respectively in the spring season at the second station, while in fish the highest values of the antibiotic (AMO) were recorded in the muscles and liver of fish 8.7 mg/ kg and 6.2 mg/kg respectively in the spring in the second station. The presence of antibiotics in the aquatic environment in these high concentrations is a source of great concern, as the study indicated that there is a need for more efforts, studies and investigation of the environmental consequences caused by antibiotics in environments other than the Shatt al-Arab.

Keywords: Pollution, antibiotics, fish, aquatic environment.

Introduction

Antibiotics are considered one of the most wonderful scientific discoveries in the twentieth century that revolutionized human and veterinary medicine. To 3% in 2000, this decrease is attributed to several reasons, the most important of which is the use of antibiotics. However, in recent years, they have been recognized as serious and active environmental pollutants due to their presence everywhere in high concentrations in surface water, groundwater, soil, sediments and animals in almost all parts of the world (1, 2, 3). Pollution with antibiotics in the environment is due to many factors, including the release of antibiotics that are not absorbed by humans and animals into the water stream or through liquid wastes resulting from hospitals. In addition, most of the remaining unused antibiotics from laboratories, pharmaceutical factories, residential and commercial areas, and hospitals are Dispose of them in the water stream (4, 5, 6). There are no local studies on antibiotic contamination except for the study (7) which dealt with the detection of antibiotics in drinking water treatment plants in Baghdad, Iraq. Where the study concluded that a relatively high concentration of antibiotics is present in the drinking water of both Al Wahda and Al Rasheed stations. There are also many international studies that dealt with the topic of antibiotic contamination, including study (8) which examined how the occurrence, sources and potential environmental risks of antibiotic contamination in the surface waters of East and Southeast Asian countries, where antibiotics were detected everywhere in the surface waters. These countries have concentrations ranging from less than 1 nanogram/liter to hundreds of micrograms/liter. And (9) evaluated the environmental risks of the antibiotics Amoxicillin, Enrofloxacin, Neomycin on the aquatic environment, as the study conducted a series of toxicity tests for these antibiotics on algae and bacteria, and the results showed that (ENR) and (AMO) are antibiotics that cause great concerns. On the environment, there is a need for more efforts, studies and investigation into the environmental consequences caused by both antibiotics. As for the antibiotic Neomycin, the study confirmed that the environment in the surrounding waters must be monitored before it can properly describe its environmental risks. (10) also studied the accumulation of antibiotics in Nile tilapia fish that fed on fortified diets with different doses of sulfamethoxazole for a period of four weeks and know the extent of their effect on the growth of Nile tilapia fish, The results showed that the accumulation of antibiotics in the muscles of tilapia fish was positively correlated with the size of the dose present in the diet provided to the fish, and the study showed that the antibiotic (SUL) led to the inhibition of growth in Nile tilapia. It also (11) studied the distribution, co-contamination and risk assessment of 17 antibiotics in mariculture farms surrounding the Yellow Sea, Northern China, where the study showed that the levels of antibiotics in the water are relatively low and the antibiotic Trimethoprim was the most prevalent, and it was detected in all water samples. (12) also detected 43 types of antibiotics in multiple sources of sewage and surface waters of Yangtze River in China, Where the study confirmed that antibiotic pollution causes great concerns due to its harmful effects on the ecosystem and human health, the results of the study indicated that Fluoroquinolones are the predominant antibiotics, contributing more than 50% in all sources. Because of the excessive and unregulated use of antibiotics in various activities, the aquatic environment was polluted with antibiotics. Therefore, the current study aimed to detect antibiotics seasonally in water, sediments and fish Planiliza abu in two selected stations in the Shatt al-Arab.

Materials and methods

Description of the Study Area

The Shatt al-Arab is one of the important rivers in Iraq, as it consists of the confluence of the Tigris and Euphrates rivers at the city of Qurna, north of the city of Basra, 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 to about 1500 m, and its depth ranges between 8-15 m, and the depths may reach more than that in some areas (13).

In this study, two stations were selected from Shatt Al-Arab to detect antibiotics in water, sediments and fish (Fig. 1). The first station is located in the center of Basra city near Al-Sadr Teaching Hospital within latitude and longitude 30°30'33"N 47°51'03 "E. The second station is located near the Salhia River, within latitude and longitude 30°30'24"N 47°51'27"E.

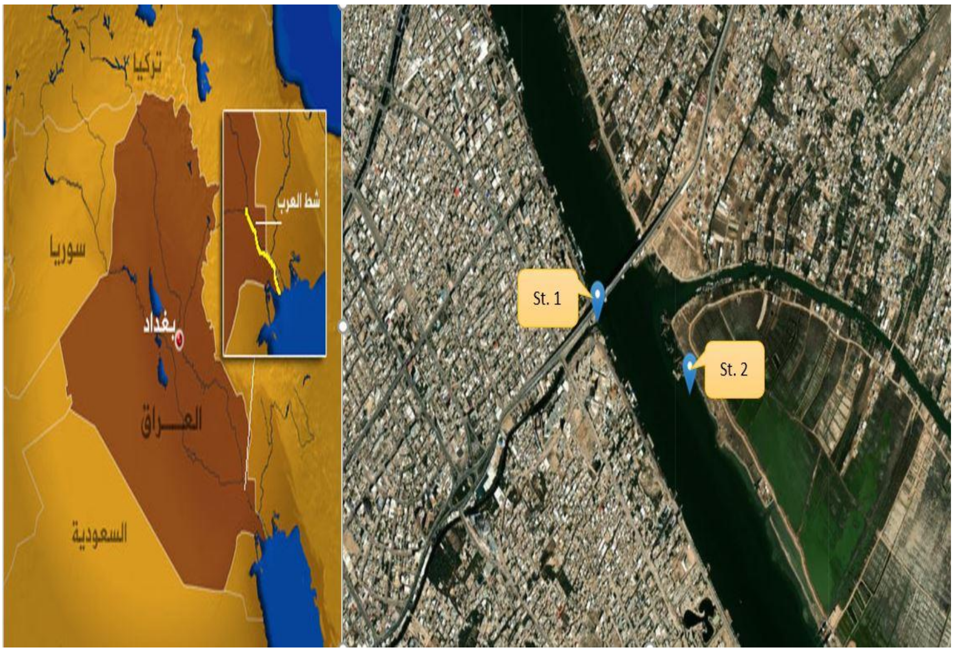


Figure (1) A map showing the two sampling stations

Samples collection

Water, sediment and fish samples were collected from the two selected study stations quarterly over a full year during the period from November 2020 to August 2021. Some environmental factors were measured during the study, including temperature (°C), pH and salinity(psu).

Water samples

Water samples were collected on a quarterly basis from the two stations using liter glass bottles that were filled to their full capacity and all were kept in a cooler box containing ice cubes until reaching the laboratory.

Sediments samples

Sediment samples were collected from the two study stations using a Grab Sampler from 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 adopted to collect the sampling fish, namely Gill net Drift, which is 120 m long and 15*15 mm in size, and the cast net has a diameter of 9 m and its holes are 15*15 mm. The caught fish are kept in a cork container. Contains crushed ice until you return to the laboratory.

Detection of antibiotics

Preparation of standard solutions

Standard solutions were prepared at aconcentration of 20 mg/L of (Amoxicillin) and 10 mg/L of (Ciprofloxacin, Levofloxacin) by dissolving the pure substances in (D.D.W) (14, 15), 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, Levofloxacin) in the sample, 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 (16). used the carrier phase consisting of (acetonitrile: methanol: phosphite bfer) 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).

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. (17). 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 / minute).

Statistical analysis

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

Results

Environmental factors

During the study period, a number of environmental factors were measured, including water temperature, pH and salinity (Table 1). The water temperature in the two study stations ranged between 15.4 °C in the fall and 31.5 °C in the summer for the first station and between 16.1°C in the fall and 31.6°C in summer for the second station, while the pH values ranged between 7.5 in spring and 7.8 in summer for the first station, and between 7.4 in autumn and 7.9 in summer for the second station, The salinity values ranged between 2.3 psu in autumn and 2.81 psu in both spring and summer for the first station and between 2.27 psu in autumn and 2.92 psu in summer for the second station.

Table (1) Environmental factors measured in the two study stations during the period from November 2020 to August 2021.

Seasons	Temp (°C)		pH		Sal (Psu)	
	St.1	St.2	St.1	St.2	St.1	St.2
Autumn	15.4	16.1	7.6	7.4	2.3	2.27
Winter	18.7	20	7.6	7.7	2.68	2.69
Spring	19.6	18.9	7.5	7.7	2.81	2.75
Summer	31.5	31.6	7.8	7.9	2.81	2.92

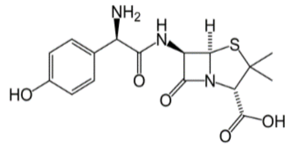
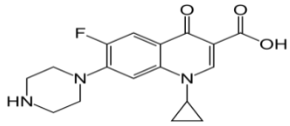
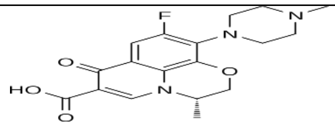
Antibiotics in water

Two groups of antibiotics Fluoroquinolone (Levofloxacin Amoxicillin) B-lactam (Ciprofloxacin,) were detected in this study (Table 2). Figure (2) shows the seasonal and local changes in the values of the antibiotics (Levofloxacin Amoxicillin, Ciprofloxacin,) in the water during the study period. The lowest values were recorded during the summer for the antibiotic (CIP)and it reached 7.48 mg/l in the first station, while the highest was in the spring for the antibiotic (CIP)It reached 24.9 mg/L in the second station.

Antibiotics in sediment

Figure (3) shows the seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, Ciprofloxacin,) in the sediment during the study period, The lowest values were recorded during the Autumn season for the antibiotic (LEV), which amounted to 9.8 mg/kg in the first station, and the highest values were recorded in the spring for the antibiotic (CIP), which amounted to 24.5 mg/kg in the second station.

Table (2) shows 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	$C_{16}H_{19}N_3O_5S$		365.40
Ciprofloxacin	Fluoroquinolone	$C_{17}H_{18}FN_3O_3$		331.34
Levofloxacin	Fluoroquinolone	$C_{18}H_{20}FN_3O_4$,		361.37

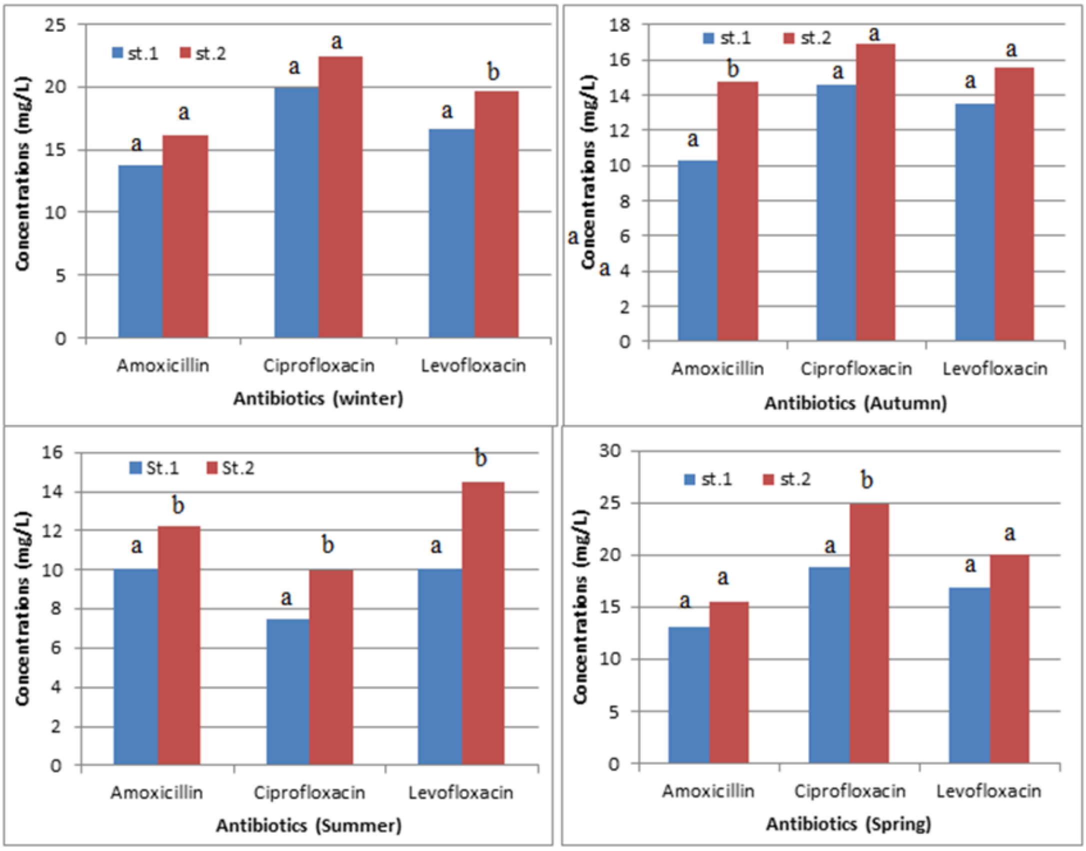


Figure (2) Seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, Ciprofloxacin,) in water during the study period, (different letters in the stations are significantly different (P<0.05).

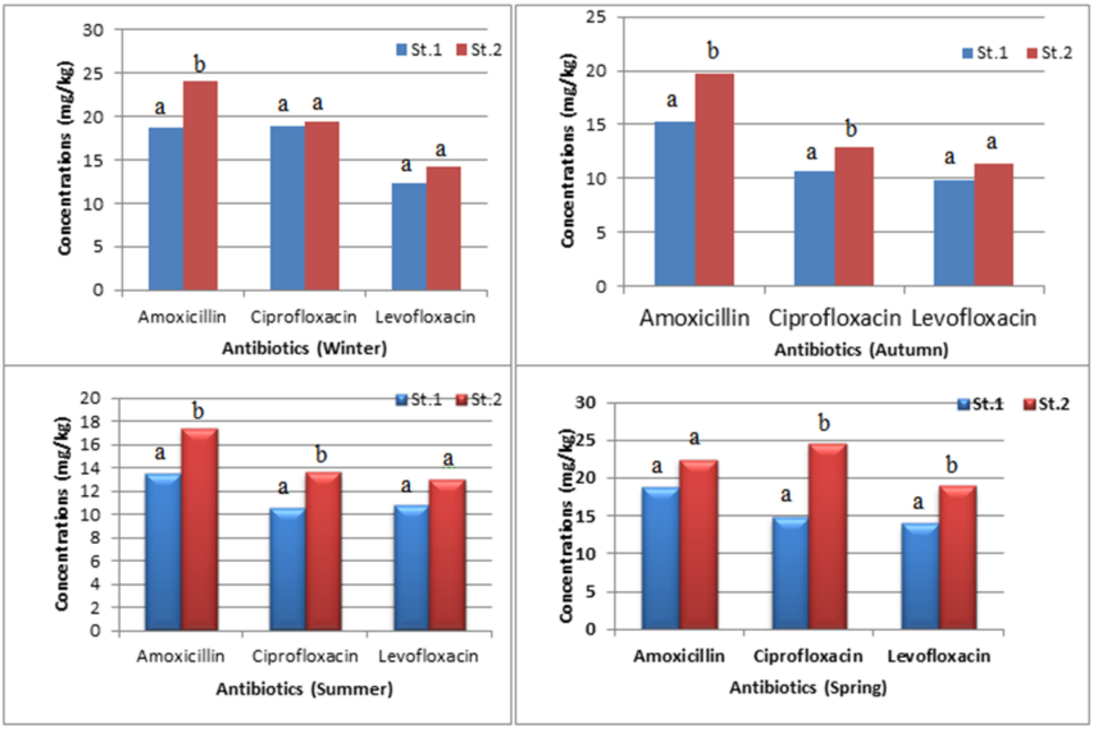


Figure (3) Seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, Ciprofloxacin,) in sediments during the study period, (different letters in the stations are significantly different (P<0.05).

Antibiotics in fish

Antibiotics in the muscles of fish

Figure (4) shows the seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, Ciprofloxacin,) in fish muscles during the study period, The lowest values were recorded during the Autumn season for (CIP) antibiotic and amounted to 2.8 mg/kg in the first station, while the highest values were recorded during the spring for (AMO) antibiotic, reaching 8.7 mg/kg in the second station.

Antibiotics in fish liver

Figure (5) shows seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, Ciprofloxacin,) in fish livers during the study period, The lowest values were recorded during the Autumn season for (LEV) antibiotic, reaching 1.3 mg/kg in the first station, and the highest in the spring for (AMO) antibiotic, reaching 6.2 mg/kg in the second station.

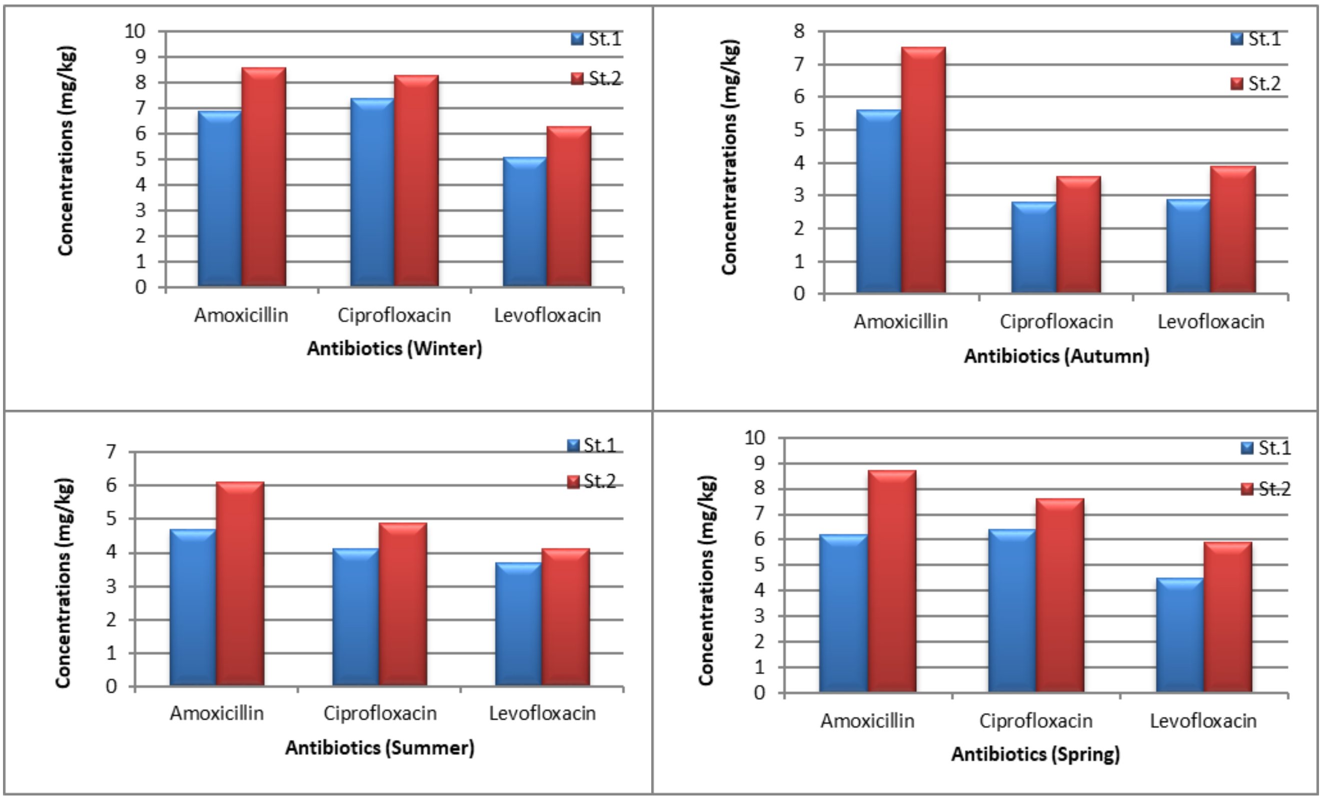


Figure (4) Seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, Ciprofloxacin,) in P. abu fish muscles during the study period.

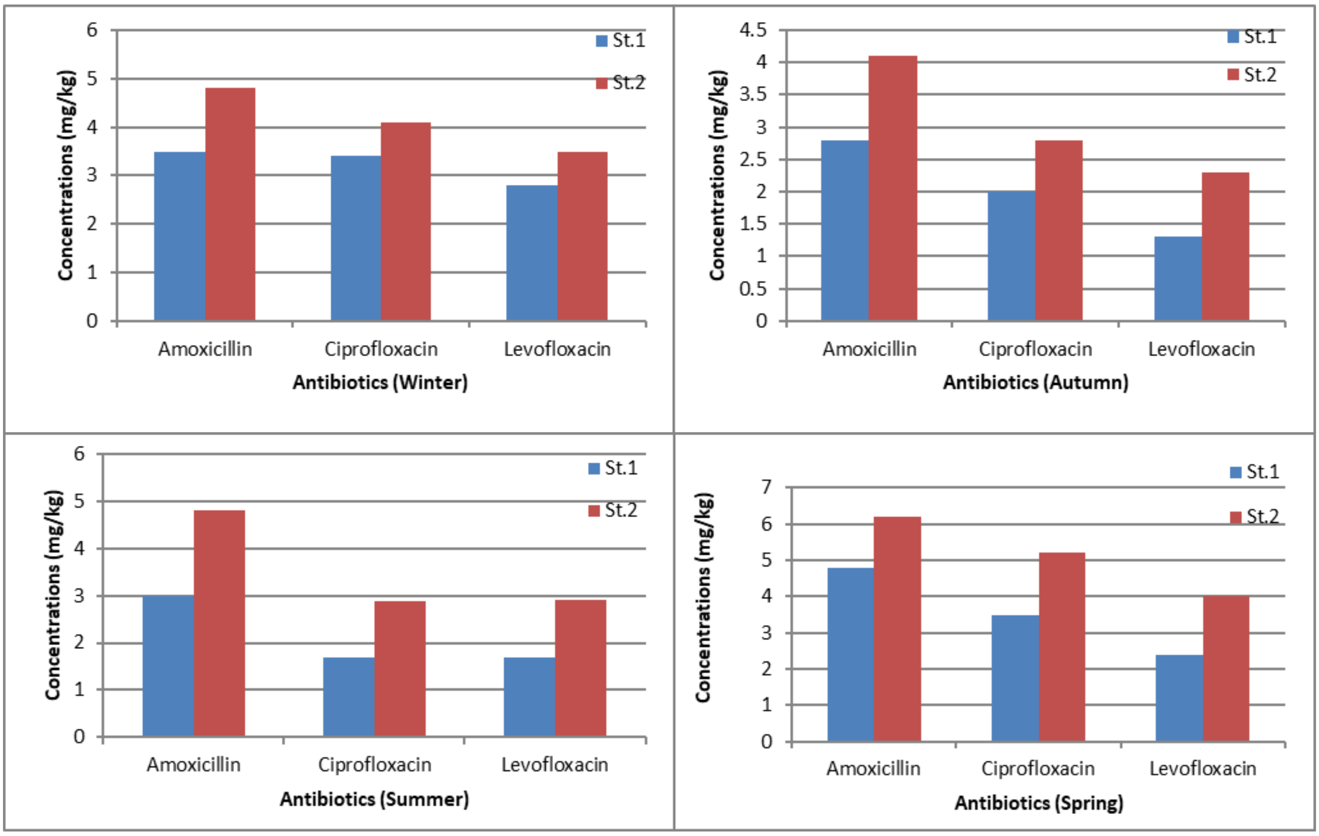


Figure (5) Seasonal and local changes in the values of antibiotics (Levofloxacin, Amoxicillin, Ciprofloxacin,) in P. abu fish livers during the study period.

Discussion

Environmental factors

Water temperature is one of the most important physical characteristics that affect the environment of water bodies and one of the most important factors that affect the chemical properties of water as well as affect the life characteristics of organisms, especially fish such as nutrition, reproduction and migration (18, 19). The results of the study showed the presence of clear changes in the values of water temperatures in the Shatt al-Arab, where the highest values were recorded in the summer and the lowest in the winter. This difference may be attributed to the nature of Iraq's climate, as it is cold and rainy in winter and hot and dry in summer (20).

The pH is a determining factor in the aquatic environment, as it affects the survival, growth and reproduction of aquatic organisms (21). The results of the pH values were within the light basal trend throughout the study period, which is a distinctive feature of Iraqi waters.

Salinity is one of the important environmental factors that affect the quality of water and aquatic organisms (22). The results of the study showed that there are slight changes in salinity values between the two study stations, and this may be due to the difference in water levels between seasons and the occurrence of mitigation due to rainfall in cold seasons and high temperature. Increasing the evaporation process in the summer, in addition to diverting the course of the Karun River into Iranian lands, which is an important tributary to support the fresh water in the Shatt al-Arab and limit the progress of the salt front (23).

Antibiotics in water

Antibiotics have been discovered in surface water, sewage, groundwater, soil and sediments all over the world. (24, 25) But this study is the first in Iraq to reveal the presence of antibiotics in the aquatic environment (Shatt al-Arab).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, this may indicate the effect of sewage that is released untreated into Shatt al-Arab water because the antibiotic (CIP) is used only to treat humans, in addition to the possibility of contamination from an animal source Because the antibiotic enrofloxacin is only used for animal treatments, it can be metabolized under certain conditions to (CIP), resulting in an increase in its concentration in water. The frequency of antibiotic discovery in the aquatic environment is mainly attributed to the extensive and unregulated use, the low human metabolic capacity, and the high stability of highly toxic antibiotics (26). As for the lowest values, they were also recorded for the antibiotic (CIP) in the summer, and this may be due to the low water level due to the high temperature and the increase in evaporation.

Antibiotics in sediment

Sediments are the largest reservoir of pollutants in aquatic ecosystems, 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 sediments act as a reservoir for pollutants, or it may be due to the tendency of most particles This was confirmed by (27) where he mentioned that the sediments are the final sink for many suspended substances in the water column, whose final fate is the sediments, as he explained. (28) The sediments are the main and final receptors for antibiotic residues. (CIP) antibiotic recorded the highest concentration in the first and second station in the winter and spring seasons compared to the other studied antibiotics. The reason for this may be due to the resistance of this antibiotic to biodegradation and water and therefore it will precipitate and settle in the sediments, while the antibiotic (LEV) recorded the lowest concentration in the sediments during the autumn season It may be due to the high susceptibility of this antibody to photo- and biological decomposition, and therefore its concentrations will decrease before it reaches the sediments.

Antibiotics in fish

This study is the first in Iraq that revealed antibiotic 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 antibiotic 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 (29).

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 antibiotic (CIP), which is considered toxic to children from the age of one day to three months (30). 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 (31).

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 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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