

Effects of antibiotic residues on some health parameters of *Planiliza abu* H. in Shatt Al-Arab, Southern Iraq

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ABSTRACT

The presence of antibiotics in the aquatic environment poses great concerns because of their impacts on water quality, aquatic organisms and human health. The current study aimed to detect the residues of antibiotics including amoxicillin (AMO), ciprofloxacin (CIP) and levofloxacin (LEV) seasonally in the muscles and liver of *Planiliza abu* fish and indicating their effects on some health aspects of fish during November 2020 through August 2021 in two selected stations from Shatt Al-Arab, Basra, Iraq. The samples were analysed using high performance liquid chromatography (HPLC). The current study is the first in Iraq to indicate the presence of antibiotic residues in fish. The study recorded high levels of antibiotics in the muscles and liver of fish, and the concentrations were higher in the second station than in the first one. The antibiotic AMO occupied the highest concentration in the muscles and liver of fish in the second station during the spring exhibiting 8.7 and 6.2 mg kg⁻¹, respectively. The lowest values of CIP were recorded in fish muscles in the first station during the autumn, amounting to 2.8 mg kg⁻¹. The lowest values of LEV were recorded in fish livers in the first station during the autumn amounting to 1.3 mg kg⁻¹. In this study, the accumulation of antibiotics in the liver and muscles of fish exhibited negative effects on the health standards. The presence of antibiotic residues in fish samples in these high concentrations is a source of great concern as it is a major source of human food. The study emphasized the need to conduct more studies to detect this type of pollution and know its negative effects on the health aspects of other types of fish.

Keywords: Pollution, Antibiotics, Fish, *Planiliza abu*, Haematological parameters.

Article type: Research Article.

INTRODUCTION

Antibiotics are substances that reduce or prevent the reproduction and growth of microorganisms that infect humans, including bacteria (Cheng *et al.* 2017; Torres *et al.* 2017). The role of antibiotics is not limited to treating infectious diseases in humans, but also in animals of all kinds (Thakare *et al.* 2020), and some are used as growth stimulants in aquaculture activities (Chen *et al.* 2018). It is considered necessary to maintain public health and the continuity of life, as the death rate at the beginning of the last century due to infectious diseases constituted over 50% of the total, while declined to 3% in 2000. This decline is attributed to several reasons, including the use of antibiotics. However, in recent years, they have been recognized as serious and active environmental pollutants due to their presence in 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). In order to protect the environment from contamination with antibiotics, the use must be subjected to control and within the permissible limits which is considered safer. In addition, public awareness should also be emphasized in order to reduce

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environmental pollution (Zhang *et al.* 2020), since the presence of antibiotics even at low levels in the aquatic environment is higher than enough to be polluted and leads to harmful effects on aquatic organisms (Lu *et al.* 2020). There are no local studies on antibiotic contamination in fish, as this study is the first in Iraq. There are also few international studies dealing with antibiotic contamination in fish, including the study of Fang *et al.* (2021) who worked on the accumulation of antibiotics in *Oreochromis niloticus* fed on fortified diets with different doses of sulfamethoxazole for a period of four weeks and indicating its effects on fish growth. Their results showed that the accumulation of antibiotics in the muscles of the fish was positively related to the size of the dose present in the diet provided to the fish. The study also showed that the antibiotic SUL led to inhibition of growth in Nile tilapia, emphasizing the need for rational and regulated use of antibiotics in aquaculture. Li *et al.* (2021) worked on the possibility of antibiotic accumulation in freshwater cultured aquatic organisms in Eastern China, so that, 12 antibiotics were detected in the muscle tissue of the cultured aquatic organisms as well as in water and sediments during the two years (2018-2019). Their results showed that most of the antibiotics recorded during the study were at a medium or low level compared to other studies in China and the world. However, slightly higher than the concentrations was recorded in the muscle tissue of these organisms. The 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, where they are dangerous. Bojarski & Witeska (2020) revealed antibiotics in the aquatic environment and their toxic effects on fish indicating that chronic exposure to antibiotics can cause physiological disturbances such as haematological changes, oxidative stress, histopathological alterations, weak immunity, metabolic disorders and general stress in the fish. In addition, 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 living symbiotically with fish. Nibamureke *et al.* (2019) also reported the effects of the antibiotic Nevirapine (NVP) on the liver of *Oreochromis mossambicus* in African surface waters under controlled conditions for 30 days. They concluded that NVP causes histological alterations in the liver cells followed by fibrosis around some veins and bile ducts, verifying that long-term exposure to NVP causes negative effects on fish health. Chen *et al.* (2015) investigated the bioaccumulation of 37 antibiotics in typical marine aquaculture farms, Southern China. The antibiotics Sulfamethoxazole, Salinomycin and Trimethoprim were widely detected in water samples with concentrations ranging between 0.4-0.8 ng L⁻¹. Erythromycin was the most detected antibiotic in sediment samples, with concentrations ranging between 0.8-4.8 ng L⁻¹.

Their results indicated that the concentrations of the antibiotics recorded in their study could cause potential risks to human health. Nunes *et al.* (2015) worked on the acute effects of exposure of freshwater fish *Gambusia holbrooki* to the tetracycline, exhibiting the extent of histological alterations in the gills and liver. The results of their study showed that the exposure of *G. holbrooki* to the tetracycline led to special histological alterations in the gills, and also in the enzymatic activity of the liver, especially the enzyme CAT. The current study also aimed to identify the effects of antibiotic residues accumulated in the liver and muscles of *P. abu* on some health parameters of the fish.

MATERIALS AND METHODS

Description of the study area

The Shatt al-Arab is one of the important rivers, consisting of the confluence of the Tigris and Euphrates rivers at the Qurna City, north of Basra, and then extends in the southeast direction for a distance of approximately 195 km to drain into the Persian Gulf, south of Faw City. The width of the river ranges from 400 m in Basra to about 1500 m beside Ras Al-Bishah. Thereafter it confluent with Karun River. Its depth ranges between 8 and 15 m, and the depths may reach higher in some areas (AL-Mahmood *et al.* 2011). The southern part of Shatt Al-Arab River suffers from tidal phenomenon as a result of the entry of the Persian 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 at Shatt Al-Arab to detect antibiotics in water, sediments and fish (Fig. 1). The first station was located in the centre of Basra, beside Al-Sadr Teaching Hospital within latitude and longitude of 30° 30' 33" N and 47° 51' 03" E. It was located beside a dock for commercial ships, and the movements of recreational boats and fishing boats were active. The other aspects of this station was the presence of many tourist restaurants that throw their waste into the river, and also its proximity to Al-Sadr Teaching Hospital, where it is considered as a source of great pollution discharging to the river. The second station was

located beside the Salhiya River, within latitude and longitude of 30° 30' 24" N and 47° 51' 27" E. The movement of recreational boats, transport and fishing boats was also active, and the area was influenced by the water coming from the Salhia River, which contributes to increasing the pollution of the area.

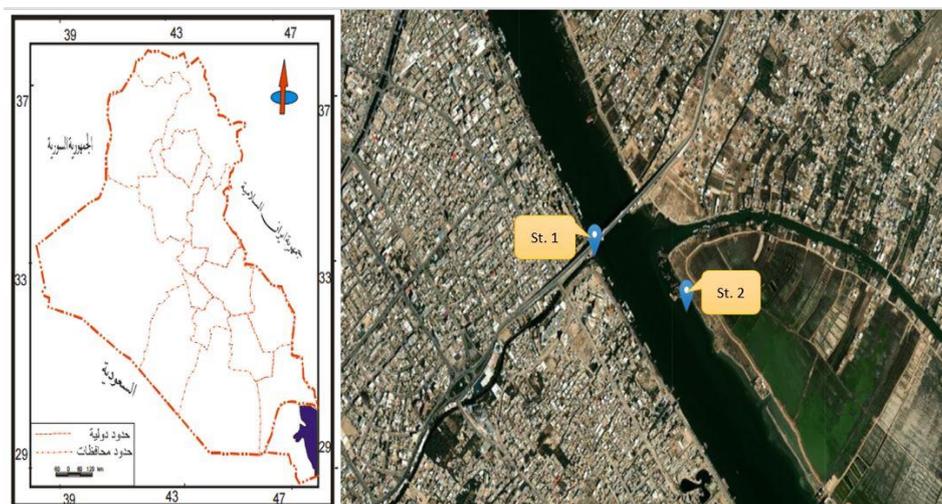


Fig. 1. A map showing the two sampling stations.

Sample collection

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

Fish samples

Two fishing methods were used to collect fish samples, the first was Gill net Drift, which is 120 m long and the size of its holes is 15 × 15 mm, and the second was cast net which has a diameter of 9 m and the size of its holes is 15 × 15 mm. The caught fish were kept in a cork container containing crushed ice until transporting to the laboratory.

Detection of antibiotics

Preparation of standard solutions

Standard solutions were prepared at a concentration of 20 mg L⁻¹ of amoxicillin (AMO) and 10 mg L⁻¹ of ciprofloxacin (CIP) and levofloxacin (LEV) by dissolving the pure substances in D.D.W (Gros *et al.* 2006; Hamscher *et al.* 2002). 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 the antibiotics (amoxicillin, ciprofloxacin, levofloxacin) in the sample, 10 g of the sample was taken and placed in a volumetric vial followed by adding a capacity of 250 mL and 100 mL (methanol: distilled water; 1:1) to it and mixing for one hour on a magnetic stirrer. Then it was placed in a sonic boom device for 30 min. Thereafter 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

Amoxicillin

The examination was conducted in the laboratories of the Ministry of Science and Technology, Department of Environment and Water, Baghdad, using a high-performance liquid chromatography device (HPLC; model

SYKAMN; Germany) according to the instruction (P1500 pump, UV2000 detector, AS3000 automatic sampling device; Unutkan *et al.* (2018). We used the carrier phase consisting of acetonitrile: methanol: phosphite buffer according to the following ratios (10:30: 60; V / V / V). Then a separation column C18 - ODS (25 cm × 4.6 mm) was used followed by using an ultraviolet detector (UV - 230 nm) at a flow rate of 1 mL min⁻¹.

Ciprofloxacin & 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; Germany) according to the instruction (Naveed *et al.* 2014). We 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 × 4.6 mm) using a radiation detector followed by ultraviolet (UV - 294 nm) at a flow rate of 1 mL min⁻¹.

Haematological parameters

Blood samples were collected by drawing blood from the heart in a test tube containing 2.5% EDTA (Ethyleneamine tetraacetic acid) as an anticoagulant.

Enzymatic tests of blood plasma

Alkaline phosphatase enzyme (ALP)

The activity of ALP enzyme in fish blood plasma was estimated using a spectrophotometer at a wavelength of 510 nm, by a ready-made laboratory kit produced by the American Company Randox. The enzyme activity was calculated according to the following equation:

$$\text{Enzyme activity (IU L}^{-1}\text{)} = \text{sample reading} - \text{control/standard reading} \times 142$$

Aspartate transferase enzyme (AST) and Alanine transferase enzyme (ALT)

The activities of AST and ALT enzymes in fish blood plasma were estimated using a spectrophotometer at a wavelength of 510 nm, by means of a ready-made laboratory kit produced by the American company Randox. The enzyme activity was calculated according to the following equation:

$$\text{Enzyme activity (IU L}^{-1}\text{)} = \text{sample reading} - \text{comparison reading} / \text{standard reading} - \text{control reading} \times 100$$

Statistical analysis

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

RESULTS

Antibiotics in fish

Antibiotics are considered dangerous environmental pollutants and spread in all sections of the aquatic environment at the present time. Two groups of antibiotics, fluoroquinolone (levofloxacin, amoxicillin) and B-lactam (ciprofloxacin) were detected in this study (Table 1).

Antibiotics in fish muscles

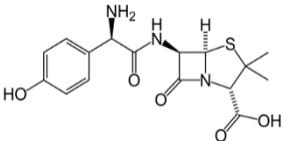
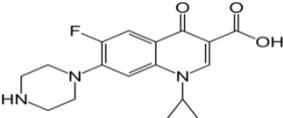
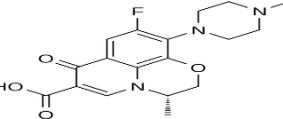
Fig. 2 shows the seasonal and local alterations in the values of antibiotics (levofloxacin, amoxicillin and ciprofloxacin) in fish muscles during the study period. The lowest values, belonging to CIP were recorded during the autumn amounting to 2.8 mg kg⁻¹ at the first station, while the highest belonging to AMO during the spring reaching 8.7 mg kg⁻¹ at the second one.

Antibiotics in fish liver

Fig. 3 shows seasonal and local changes in the values of antibiotics (levofloxacin, amoxicillin and ciprofloxacin) in fish livers during the study period. The lowest values belonging to LEV were recorded during the Autumn

season, reaching 1.3 mg kg^{-1} at the first station, while the highest belonging to AMO in the spring, reaching 6.2 mg kg^{-1} at the second one.

Table 1. The aggregates, chemical structure and molecular formula of the antibiotics (amoxicillin, ciprofloxacin and levofloxacin) to be detected using the HPLC device.

Antibiotics	Antibiotic class	Formula	Chemical structures	Mol. Wt (g mol^{-1})
Amoxicillin	B-lactam	$\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S}$		365.40
Ciprofloxacin	Fluoroquinolone	$\text{C}_{17}\text{H}_{18}\text{FN}_3\text{O}_3$		331.34
Levofloxacin	Fluoroquinolone	$\text{C}_{18}\text{H}_{20}\text{FN}_3\text{O}_4$		361.37

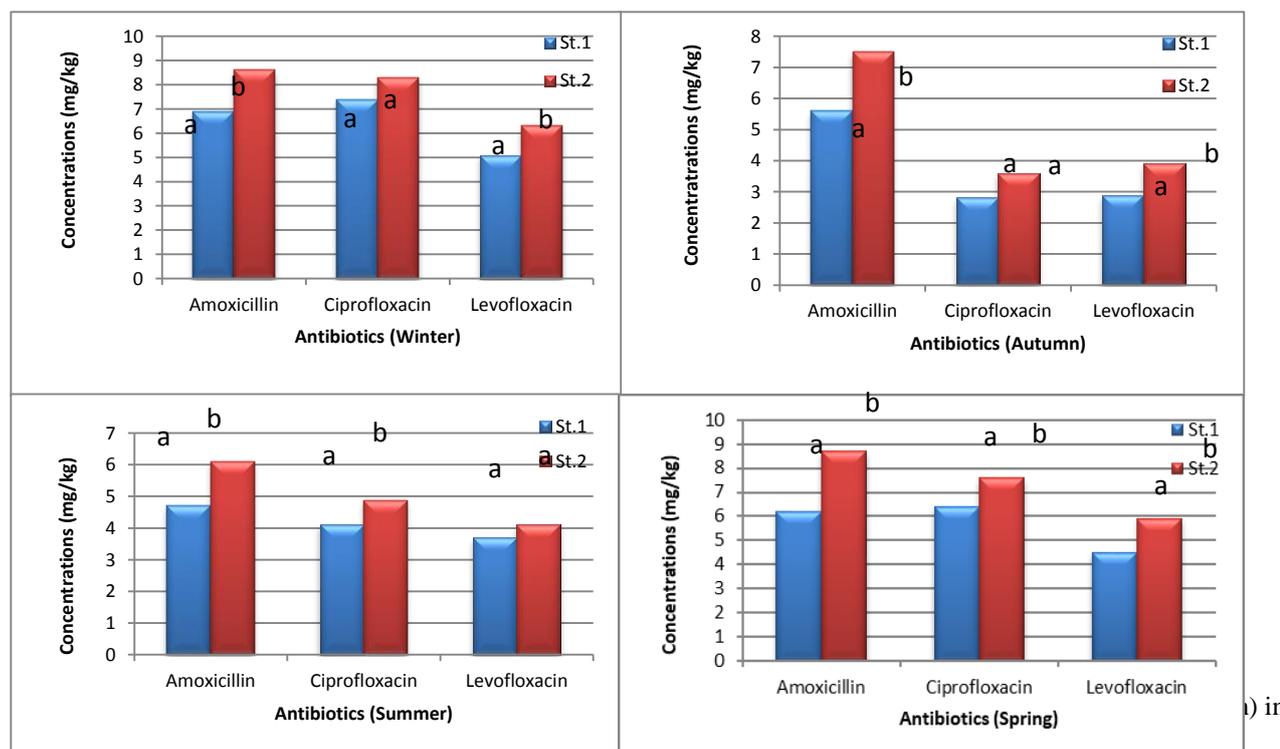


Fig. 2. Seasonal and local alterations in the values of antibiotics (levofloxacin, amoxicillin and ciprofloxacin) in *P. abu* muscles during the study period.

Haematological parameters

Enzymatic tests of blood plasma

Table 2 depicts the seasonal and local alterations in the concentrations of alkaline phosphatase, Aspartate transporter, and alanine transporter in *P. abu* during the study period. The lowest value of alkaline phosphatase were recorded at the first station during the spring amounting to $52,736 \text{ IU L}^{-1}$, while the highest at the second station during the summer (77.49 IU L^{-1}).

In the case of the lowest value of aspartate transporter, it was recorded at the first station in the winter amounting to 43.83 IU L^{-1} , while the highest at the second station during the summer ($65,343 \text{ IU L}^{-1}$).

The lowest values of alanine transporter were recorded at the first station during the winter (3.8 IU L⁻¹), while the highest at the second station during the summer (6.536 IU L⁻¹).

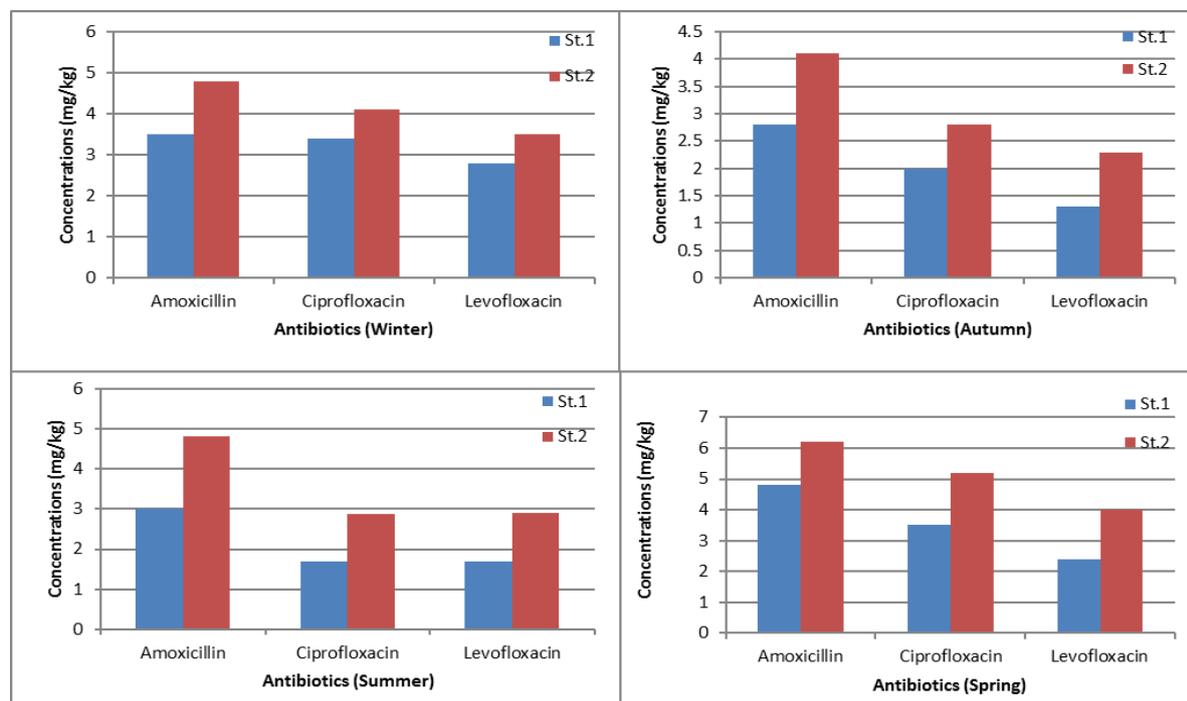


Fig. 3. Seasonal and local alterations in the values of antibiotics (levofloxacin, amoxicillin and ciprofloxacin) in *P. abu* livers during the study period.

Table 2. Concentrations of alkaline phosphatase, aspartate transporter, and alanine transporter in the blood plasma of *P. abu* at the two study stations.

Haematological parameters (IU L ⁻¹)	Seasons (mean ± standard deviation)							
	Autumn		Winter		Spring		Summer	
	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2
Alkaline phosphatase (ALP)	62 ± 1.322 ^a	71.566 ± 4.331 ^b	58.5 ± 3.122 ^b	67.84 ± 5.063 ^b	52.736 ± 2.964 ^a	67.6 ± 1.276 ^b	70.746 ± 1.092 ^b	77.49 ± 3.350 ^a
Aspartate transporter enzyme concentration (AST)	46.673 ± 5.507 ^b	56.08 ± 5.181 ^b	43.833 ± 1.258 ^a	54.783 ± 3.986 ^b	51.206 ± 7.806 ^b	59.656 ± 0.904 ^a	50.703 ± 3.762 ^a	65.343 ± 4.737 ^b
Alanine transporter enzyme concentration (ALT)	4.2 ± 0.3 ^a	6.266 ± 0.321 ^a	3.8 ± 0.435 ^a	5.533 ± 0.472 ^a	4.706 ± 0.352 ^b	6.303 ± 1.108 ^b	4.416 ± 0.422 ^a	6.536 ± 0.890 ^a

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

DISCUSSION

Antibiotics in fish

Fish were used as bio-indicators to determine 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. This study is the first to measure antibiotic concentrations in fish, as antibiotics were detected seasonally in the muscles and liver of *P. abu* during a whole year. The results of the current study showed a large variation in the values of antibiotics in the muscles and liver of fish between high and low. The results also showed that most of the concentrations of antibiotics recorded in the current study are very high and considered to be of high risk. In addition, it can have direct health risks to humans in different age groups, especially CIP, since its low concentration is considered toxic to children from the age of one to three months (Cui *et al.* 2018). Hu *et al.* (2010) and Kemper (2008) reported that the presence of antibiotics in the aquatic environment, even at very low concentrations, leads to

chronic and hidden effects, such as the effects on fish behaviour and the feminization of males of some fish species. The concentrations of antibiotics were higher at the second station than at the first one, which may be due to its vicinity to the Salhia River, since it is polluted with sewage. The results of the study also showed that the accumulation of antibiotics was higher in the fish muscles than in the liver, which may indicate the persistence of this type of pollution inside the fish body, reflecting that 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; Abbaszadeh & Şişman 2021; Jorfipour *et al.* 2022). The highest values of AMO were recorded in the liver of *P. abu*, as well as high values in the muscles which may be due to its high ability to accumulate and its high presence in the environment as a result of its very wide use in the treatment of humans and animals. It is a broad-spectrum antibiotic used to treat many infections, caused by many types of bacteria (Bielen *et al.* 2017). In the case of LEV, it was recorded in low concentrations in the fish liver and muscles which may be due to its rapid chemical breakdown.

Haematological parameters

Enzymatic tests of blood plasma

Blood parameters are important indicators in determining the health status of living organisms, including fish (Gharaei *et al.* 2016). There are important indicators that can express the internal state of the body to exhibit a picture of the positive or negative physiological and immune responses in fish (Akbari & Jahanbakhshi 2017). Data on the haematological and immune response in fish after exposure to antibiotics are scarce and ambiguous, since they depend on the antibiotic dose and the sensitivity of different fish species. The enzymes (ALP, ALT and AST) are among the most important digestive enzymes present in the organism, especially the liver tissues. The alkaline phosphatase is widespread in the body, as it is found in the liver, kidneys and intestines and also helps in the bone calcification processes, as well as helping intestinal cells to absorb fats and transport inorganic phosphorous (Abedi *et al.* 2013). The level of the enzyme rises in case of acute and chronic liver diseases such as hepatitis viral, renal dysfunction and bone disease (Huang *et al.* 2006). It elevates its activity when exposed to high pollutants (Rajamanickam 2008). As for the aspartate transporter enzyme, it belongs to the non-functional plasma enzymes, which are usually inside the cells of the liver, heart, muscles, kidneys, gills and other organs. It is released from the cells in case of damage. It is the most specific enzyme for liver problems and high levels of it are observed in hepatitis (Rajamanickam 2008). In the case of the alanine transporter enzyme, it is secreted mainly in the liver, despite its presence in other tissues of the body. However, its large quantities are made in the liver (Robert, 2011) and therefore its activity is measured to diagnose many liver injuries as well as acute and chronic diseases (Huang *et al.* 2006). The results of the current study showed alterations in ALP, AST and ALT during the study period. It was observed that the base phosphatase, aspartate transporter and alanine transporter were found at the second station during the summer. Coppo *et al.* (2001) pointed out that the increased AST, ALT and ALP release occurs in acute and chronic liver disorders. These enzymes are vital indicators of acute liver damage and can therefore serve as a diagnostic tool for assessing liver cell necrosis. The liver is one of the main target organs for antibiotic side effects in fish (Limbu *et al.* 2018). Previous studies indicate that the activities of hepatic enzymes, ALT and AST in fish are often raised by exposure to antibiotics, which may indicate the toxic effect of antibiotics on the liver in fish (Hoseini & Yousefi, 2018; Nakano *et al.* 2018)

CONCLUSION

1. The study recorded a significant increase in the level of antibiotic contamination in *Planiliza abu* fish, which may be due to the absence of environmental control and the release of pollutants in general and sewage in particular.
2. The second station recorded high concentrations of antibiotics compared to the first one.
3. The results of the study showed that the concentrations of amoxicillin were high during the study period.
4. Concentrations of antibiotics were higher during the spring than during the other seasons.
5. The study showed that the presence of antibiotic residues in fish had negative effects on the health standards.

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