EFFECT OF LEAD AND CADMIUM ON HEMATOLOGICAL PARAMETERS AND LIVER ENZYMES ACTIVITY IN *Luciobarbus xanthopterus* (Heckel, 1843) IN TIGRIS River

Z. M. Abbas	L. M. Abbas	J. S. AL-Sariy
Lecturer	Senior Scientific Researcher	Prof.
Coll. Sci. Univ. Wasit	Agr.Res.Cen\ Cie.Res.Com.	Coll. Sci., Univ. Wasit

Correspondencezmabbas@uowasit.edu.iq

ABSTRACT

The current study was conducted in two different sites , these, sites S1 and S2 were located before and After Al- Kut Barrier respectively at Tigris River middle of Iraq. This study focused on evaluation the effect of lead Pb and cadmium Cd on Guttan, *Luciobarbus xanthopterus* for the period from January to September 2021. Monthly, some of water qualities were measured. The values for Water temperature ranged between 6.2 to 20.6 ºC , DO_2 were recorded values between 11.16 to 6.12 mg /l. , BOD_5 values were showed values between 1.03 to 3.42, water turbidity values of 11.7 to 72.4 NTU, The pH water values were ranged between 6.4 to 7.7 at sites S1 and S2 respectively. Lead and Cadmium concentration of water at Tigris River were 0.010 at S1 to 0.513 µg /l at S2 for Lead .Where's, cadmium concentration ranged between 2.53 at S1 to 11.573 µg /l at S2. At the present study, some of Liver enzymes activity for Guttan were measured. These fishes blood enzymes AST, ALT and ALP were recorded a value of 51.2, 15.53 and 8.98 U/l respectively at S1. And a values of 410, 47.52 and 72.89 U/l for the same enzymes respectively at S2.The conclusion of current study showed that water quality at S1was suitable for Guttan, as well as, Fishes were best healthy than these at S2.

Key words: fish, blood, Pollution, life below water, food safety

تأثير الرصاص والكادميوم على الصفات الدمية والنشاط الانزيمي للكبد في اسماك *Luciobarbus xanthopterus* (Heckel, 1843) في نهر دجلة / العراق

زينب محمد عباس	لؤي محمد عباس	جميل سعد السراي
مدرس	رئيس باحثين علميين	استاذ
كلية العلوم/جامعة واسط	مركز الثروة الحيوانية والسمكية	كلية العلوم/جامعة واسط

المستخلص

اجريت الدراسة الحالية في موقعين مختلفين هما، S1 و S2 قبل وبعد سدة الكوت على التوالي عند نهر دجلة. ركزت الدراسة على تقيم تاثير عنصري الرصاص Pb والكادميوم Ca في اسماك القطان *Luciobarbus xanthopterus* للمدة من كانون الثاني لغاية ايلول 2021. شهريا تم قياس بعض خواص نوعية المياه، وكانت قيم المديات تراوحت لدرجة حرارة المياه بين 6.2 الى 20.6 م° ، سجلت قيم الاوكسجين الذائب قيما بين 11.16 الى 6.12 ملغم/ لتر، اظهر المتطلب الحيوي للمياه قيما بين 1.03 الى 3.42 ، وقيم العكارة كانت بين 11.7 الى 72.4 نفثالين وحدة كدرة، وتراوحت قيم حموضة المياه بين 6.4 الى 7.7 عند الموقع S1 و الموقع S2 على التتالي. تراوحت تراكيز العناصر المدروسة عند سدة الكوت في نهر دجلة بين 0.010 عند S1 الى 0.513 µg/ لتر عند S2 لعنصر الرصاص ، بينما تراوحت المديات للكادميوم بين 2.53 عند الموقع S1 الى 11.57µg / لتر عند الموقع الثاني. قيست في الدراسة الحالية انشطة بعض الانزيمات في اسماك القطان *Luciobarbus xanthopterus* ، وسجلت انزيمات الدم هذه نشاطا في دم الاسماك AST و ALT و ALP قيما تراوحت 51.2 و 15.53 و 8.98 U/l على التتالي عند الموقع S1 ، وقيما 410 و 47.52 و 72.89 U/l للانزيمات نفسها على التوالي عند الموقع S2 . تستنتج الدراسة الحالية بان نوعية المياه عند الموقع كانت اكثر ملائمة للاسماك، فضلا عن ان الاسماك عند الموقع S1 افضل حالا من الناحية الصحية مقارنة بالموقع S2 .

الكلمات المفتاحية: أسماك ، دم ، تلوث، الحياة تحت الماء، سلامة الغذاء

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INTRODUCTION

Fish is one of the most important sources of animal protein, which contains essential amino acids and a number of minerals and salts. For this reason, fisheries have become of increasing importance and interest by scientists and researchers in order to develop and preserve them as part of the requirements of providing food in a balanced manner with the increase in the world population (20). Rivers are exposed to pollution with heavy metals from various sources such as domestic and industrial waste, mining activities and agricultural activities, including the addition of fertilizers and pesticides, which affects the ecological balance in water systems (16). Fish have the ability to accumulate heavy metals in a higher concentration than in water and sediments due to their feeding on algae and small organisms, as well as on organic matter in the aquatic environment (6). Therefore, fish have been used in particular for detection or as a basic guide and indicator to know the presence of pollution to assess the health of the aquatic environment (29). The commercial importance of Gattan fish, as many local studies were conducted on them in many Iraqi water environments, including Tigris and Euphrates rivers, lakes and reservoirs. Where many studies have been conducted to estimate the concentrations of heavy metals in different organs in Gattan and effect of heavy metal accumulation on liver enzyme activity (5,8,25). The current study aims to measure some physical and chemical properties at the Kut barrier in the Tigris River and to compare the two sites before and after the Kut barrier and to measure the extent of two heavy metals pollution in the two sites and the impact of pollution on the activity of liver enzymes in *Luciobarbus xanthopterus*.

MATERIALS AND METHODS

The study was carried out on the Tigris River within the city of at Al- Kut Province/ Iraq (Figure 1) for the period from January to September 2021. Two different sites were chosen. The first site (S1) is 3 km north of the river. It is characterized by a lack of fishermen and industrial activity. Residential homes and government institutions are spread on both sides of the river. It is characterized by slow water flow in the river. The second site (S2) is 3 km south of the river. It is characterized by river fishing, industrial and recreational activity. Wide housing complexes and agricultural lands are spread on the right side of the river, while on the left side are markets, cafes and agricultural lands , and the reed plants *Phragmites austrail*s is abundant. Gattan fish caught using two types of gill nets (100 x 3 m), with a mesh size of 2.5 and 3.5 cm. As well as the cast net, with a length of 2 and 2.5 cm. A total of 64 fish samples were collected for each of the *Luciobarbus xanthopterus* fish in total lengths ranging from 33.39 to 57.67 cm with a total weight ranged between 491.5 to 1949.6g. Water samples were collected during the low temperature season represented in January, February, March and April, while the hottest months are June, July, August and September from the two study sites for the year 2021. Water samples were taken in the morning from the surface layer about 20 cm deep. Physical and chemical measurements of water which included: water temperature measured using mercury thermometer from 0-55 °C (10). pH for water by pH meter 180Mi- Italy (4, 7, 10). Water turbidity (NUT) measured by turbidity meter (2100N, USA). Dissolved Oxygen (mg/l) was measured using Winkler's method of Modification Azid described by APHA (11,13) and BOD_5 values were calculated through the following equation:

$$BOD_5 \text{ mg/L} = \text{Primary Dissolved Oxygen} - \text{Final Dissolved Oxygen}$$

Blood samples

Blood samples were obtained from the caudal vein of the fish using a 3 ml syringe. Serum were separated from blood samples in a centrifuge at 3000 rpm for 10 minutes, placed in sterile tubes and kept in freezing at -20 ºC to analyze the biochemical indicators in the serum, which include liver enzymes, which are ALP, AST and ALT.

Measurement of liver enzymes activity

Measurement of AST and ALT enzymes using a kit from the Italian company Assel. The enzymes were measured according to the method of (30). Alkaline phosphatase enzyme ALP was measured according to the (12) using a kit of the French BioLabo company with a wavelength of 510 nm. The analyzes were carried out using the VEGASYS Chemical Analyze from company (AMS Co., Italy).

Measurement of Lead and Cadmium in water samples

Other water samples were collected, filtered, and kept in 5 mL HNO3/L (55%) water to prevent mineral adsorption on the inner surface of the bottle and stored at 4°C for heavy metal determination. The concentrations of heavy metals under study were estimated according to APHA (12).

Statistical analysis

The statistical program SPSS version 20 was used to analyze the data and the data was analyzed according to the

Randomized Complete Block Design (RCBD), and then the significant differences between the means were compared with Duncan's polynomial test Duncan Multiple Range to find the significant differences between the different treatments at a significant level ($P \leq 0.05$) (17).

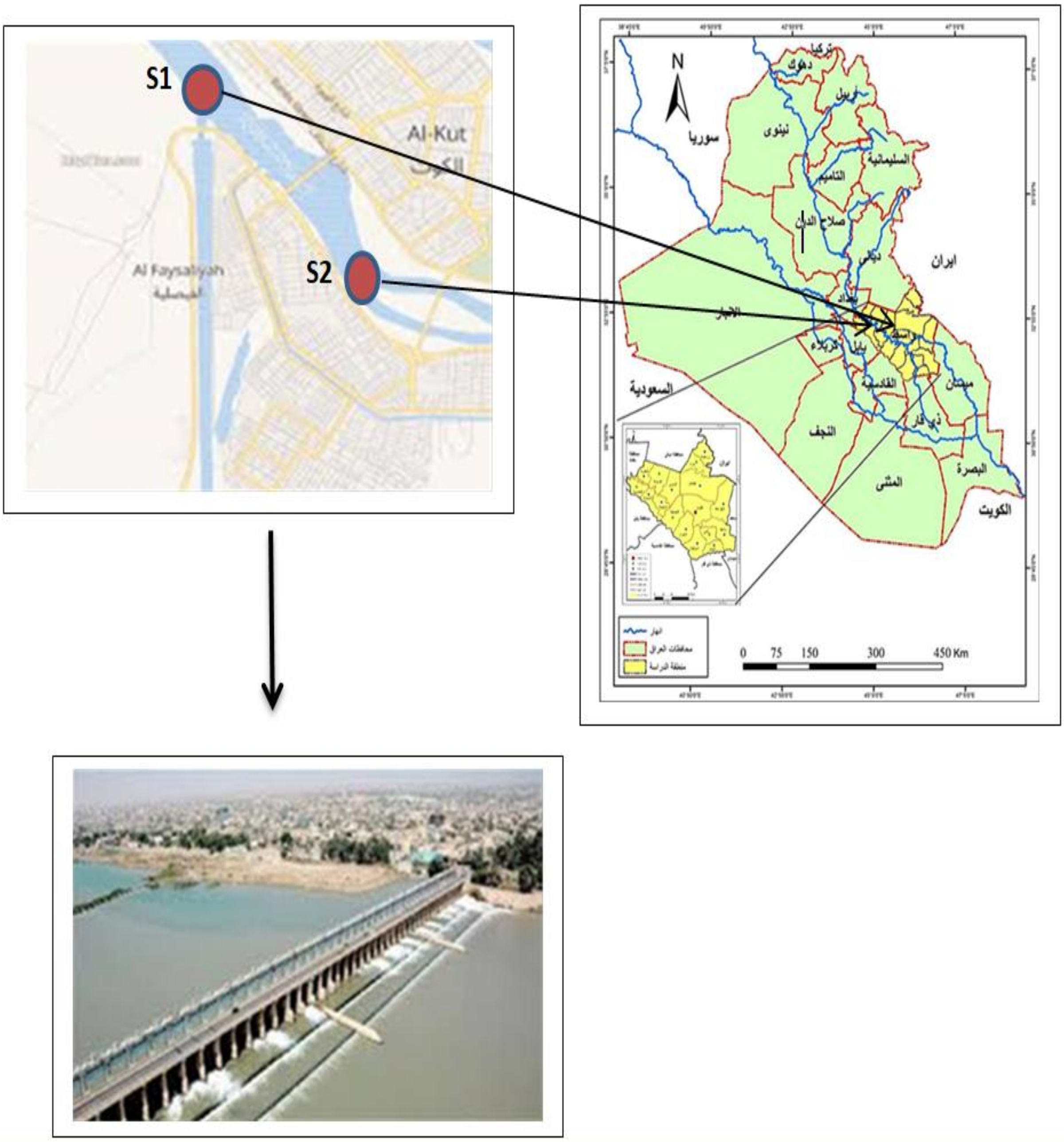

Figure 1. Map representing the two sites of the current study at the Kut barrier in the Tigris River / Wasit Governorate / Iraq

RESULTS AND DISCUSSION

Water physical and chemical characteristics: Water temperature is an important environmental factor affecting behavior, growth, metabolism, immune response, and survival of fish (18). The fluctuation in the temperature of the effluent entering the river (4). Tables 1 and 2 indicate air temperature fluctuation during the study period, ranging between 48.1 °C in August and 18.2 °C during January 2021in first site (before the barrier) , while the air temperature values in the second study site (after the barrier) ranged between 47.3 °C recorded in July and 19.2 °C during January 2021. The water temperature in the first study site ranged between 20.63 ºC in August and 6.43 ºC in February, while the temperature of the water in the second site was between 20.57 ºC in August and 6.23 ºC in January. The results of the current study, showed a fluctuation in the water temperature values during the months of the year and there are no significant differences $P \leq 0.05$ in the values between the two studied sites. The temperature rises during the hot months and decreases during the cold months, which indicates that the water of the Tigris River is affected by changes in climate temperatures. As Mustafa *et.al* (25) indicated that most environmental studies of Iraqi waters bodies in general and the Tigris river in particular are characterized by high temperatures during the summer months and lower temperatures during the winter months. The current study agreed with the study of Al-Aboudi *et.al* (7) recorded an the sun's rays are the controlling factor in temperature, in addition to the fact that the location and weather conditions are the same as they are under the influence of one climate, as evidenced by an increase in the water temperature before and after the Kut barrier during the hot months and a decrease in the water's temperature during the cold months of the year 2015.The results of Table 1 and 2 showed that pH valus with as light during the different months and at the two sites throughout the study period. The values ranged between 6.4 to 7.6 for the first site and 7.2 to 7.7 for the second site. This results within acceptable limiting for the Iraqi environment, which range between 6.5-8.5. The pH of a river depends on the geological level of the river bed and environmental and human influences (15). The correlation between the concentration of carbon-free hydrogen ions produced by the breakdown of bicarbonates and the automatic regulation capability of water bodies like rivers and lakes is crucial (22). The results of the current study are consistent with the results of the study of Radhiy and Abbas (28), where the values of the acidity function ranged between 7.5-7.8 in their study at the Kut barrier in the Tigris River in central Iraq. The results of the current study were similar to previous studies, including the study of Rhadi *et.al* (27) in a study in the Tigris River at the Kut barrier, which was between 6.8 to 7.9, and the study of AL-Sarraj.*et.al* (9) in the Tigris River within the city of Mosul. Water turbidity results from suspended particles such as clay and silt, chemical sediments such as manganese and iron, and organic particles such as plant and organism remains (19). Turbidity, which has an inverse relationship to transparency, is a measurement of all the chemicals that are suspended in water. Clay, greens, germs, animals and plants are only a few examples of the precise materials floating in water that prevent light from passing down the water column, causing refraction and dispersion (34) . The results of Tables 1 and 2 indicated that there were a significant differences ($P \leq 0.05$) for turbidity values between the two studied sites and between the months of the current study. The lowest value for the first site was 11.7 NTU during January, and the highest value for the second site was 72.4 NTU August. The current results showed that, the high turbidity values in the hot months and their decline in the cold months, the turbidity values of the second site rose in comparison to the first site throughout the study's months. The study of Rhadi *et.al* (27) in the Tigris River at the Kut barrier recorded higher values of turbidity than what was recorded in the current study, and the increase in values was during the month of July 2010 and the decrease in the values during November 2009, and values ranging between 30-78 units were recorded as naphthalene turbidity after the Kut barrier in comparison. Results pointed out that the values before the Kut barrier and attributed the reason to the positive effect of the barrier in reducing the turbidity values in the lower parts of the river, and this may not be consistent with the current results. The study of Al-Temimy (3) showed that the decrease in turbidity values during the cold months and its rise during the hot months was associated with the temperature variation of the hot flows of the Musayyib power plant for power generation throughout the study period. Dissolved Oxygen and Biological Oxygen Demand (BOD_5) as a gas has a low solubility in water, in addition to the fact that the amount of oxygen in the water varies with temperature and salinity (33). The results of Tables 1 and 2 recorded the highest value for the first site recorded 11.16 mg/liter during December and the lowest value attained 6.63 mg/liter during August 2021, and the highest value for the second site was recorded 9.70 mg/liter during January and the lowest value recorded 6.12 mg/liter during September . The decrease in dissolved oxygen values in the second site may be due to the large expenditures of clubs, recreational places, cafes and markets, as well as residential and slum areas and factories on both sides of the river, which leads to increase in the dumping of waste loaded with organic materials into the river, which leads to increase in the demand for oxygen by microorganisms. Thus, the first site has good aeration, the results of the current study agree with the study of Abbas (1, 2) which indicated the good aeration of the waters of the Tigris River before the Kut and Hindyah barrier. An increase in dissolved oxygen values in the cold months and a decrease in the hot months may be due to changes in temperature (32)**.** BOD_5 is a measure of the amount of oxygen required or consumed by bacteria and microorganisms under aerobic conditions during the biological oxidation of the organic materials present in the waterway (14). BOD_5 is an important factor to know the water quality because it mainly affects the concentration of dissolved oxygen in the water, as the numbers of the values of the biological oxygen demand indicate the deterioration of the water quality, while the values for natural water range between 0-2 mg/liter (31). The results of Tables 1 and 2 showed that the BOD_5 ranged from 1.03 to 2.85 mg / L for the first site and between 1.19 to 3.42 mg / L for the second site. The reason for the high values of the BOD_5 in the site after the barrier may be due to the disposal or disposal of sewage water to it, which represents polluted water, a container of many organic compounds, which is a major source of microorganisms that decompose these materials and pollutants, and then decrease the amount of dissolved oxygen and thus The values rise compared to the first site far from the sources of pollution from the sewage flow and the rise in the water level due to storage, which has a significant impact on reducing the organic content of water. As for the decrease in water levels during the hot months, it concentrates the organic pollutants that consume oxygen and leads to an increase in the values of the vital oxygen requirement. The increase in temperature leads to an increase in The activity of microorganisms that decompose organic matter, which leads to more consumption of oxygen (24).

Table 1. Values of some physical and chemical properties of the waters in first site.

Months	Air temperature (C◦)	Water temperature (C◦)	PH	Turbidity (NTU)	DO (mg/ L)	BOD_5 (mg/ L)
January	18.17	6.70	7.3	11.7	11.16	2.33
February	20.00	6.43	7.4	15.1	10.77	1.03
March	25.00	9.10	7.6	15.5	10.67	2.27
April	30.17	12.30	7.6	16.8	9.43	2.32
June	39.93	17.40	7.4	20.4	9.88	2.30
July	44.97	20.47	7.4	26.5	9.20	2.71
August	48.10	20.63	7.6	41.2	6.63	2.62
September	41.10	20.00	6.4	24.6	7.48	2.85
Range	18.17-48.1	6.43-20.63	6.4-7.6	11.7-24.6	6.63-11.16	1.03-2.85
Mean ± S.E	33.43±4.1	14.1±2.1	7.3±0.1	21.4±3.3	9.1±0.57	2.3±0.1

Table 2. Values of some physical and chemical properties of the waters in second site.

Months	Air temperature (C◦)	Water temperature (C◦)	PH	Turbidity (NTU)	DO (mg/ L)	BOD_5 (mg/ L)
January	19.17	6.23	7.2	26.8	9.70	1.19
February	22.00	6.33	7.5	38.2	8.57	2.50
March	27.13	9.60	7.6	45.4	7.37	2.70
April	32.17	10.17	7.5	49.7	7.72	2.76
June	43.93	17.73	7.6	56.6	7.92	2.33
July	47.30	19.83	7.7	64.5	8.94	3.24
August	47.10	20.57	7.5	72.4	6.12	2.94
September	43.10	19.83	7.2	59.6	6.33	3.42
Range	19.1-47.3	6.23-20.57	7.2-7.7	26.8-59.6	6.12-9.7	1.19-3.42
Mean ± S.E	35.2±4.0	13.7±2.2	7.4±0.06	51.65±5.2	7.8±0.4	2.6±0.2

Lead and Cadmium concentrations in water: The results of Tables 3 showed significant differences (P ≤ 0.05) in the concentration of Lead and Cadmium in water between the two studied sites. The concentration values of Lead in the waters of the Tigris River recorded between 2.5 to 9.8 µg / L for the first site and between 3.3 to 11.5 µg / L for the second site, while Cadmium concentrations in the water of the Tigris River were recorded between 0.013 to 0.173 µg / L for the first site and between 0.2 to 1.1 µg / L for the second site. The results showed increase in the concentration of Lead and Cadmium in the second site, and it was higher than the permissible limits in surface waters set by the World Health Organization, 0.01 and 0.005 µg / L respectively (37). The variations in Lead concentrations between the two sites before and after the barrier may be due to the effect of the difference in the volume of pollutants carried by the water from the cities through which the river passes and waste water being thrown in abundance continuously and without treatment. The high values of lead concentrations in the second site after the Kut barrier in the Tigris River may be associated with industrial and population activity, especially sewage expenditures on both sides of the river. Dust storms and dust may increase the transport of heavy metals such as Lead, as well as agricultural activities and soil washing that may increase water pollution and lead accumulation, especially in the summer (35). The high concentration of Cadmium in the waters of the Tigris River during the hot months is consistent with what was observed by Joudah and Fahad (20), who recorded a decrease in the concentration of the same element during the winter months and a rise during the summer months in the Gharraf River. The element is due to the evaporation factor as a result of high temperature.

Table 3. Concentrations of Lead and Cadmium (µg/L) in water at Kut barrier in the Tigris River for the period from January to September 2021

Month	Pb µg/L		Cd µg/L	
	S1	S2	S1	S2
January	0.013Bb	0.220Ad	9.887Ba	7.120Ab
February	0.080Bb	0.260Ad	6.017Bb	11.573Aa
March	0.087Bb	0.310Ac	4.130Ad	4.510Ae
April	0.010Ab	1.127Ae	4.713Bd	6.823Ac
June	0.057Bb	0.240Ad	6.440Ab	6.073Ac
July	0.173Ba	0.303Ac	5.310Ac	5.673Ad
August	0.140Ba	0.427Ab	2.537Be	4.543Ae
September	0.147Ba	0.513Aa	3.360Be	3.353Ae
Range	0.013-0.173	0.2-1.1	2.5-9.8	3.3-11.5
Mean ± S.E	0.08±0.02	0.4±0.1	5.2±0.80	6.2±0.88

Different capital letters indicate significant differences within the same row locally at the probability level (P ≤0.05). Different lowercase letters indicate significant differences within the same column per month at the probability level (P ≤ 0.05).

Measuring the activity of some liver

Enzymes: Liver enzymes are biomarkers of great interest to researchers in environmental or applied studies because they are reliable, rapid and widely applicable to many animals including fish and other tissues such as kidneys, brains, livers and gills (23). Average values of liver enzymes in the blood of Gattan caught from the Tigris River at the Kut barrier between the two studied sites and throughout the study months. The lowest values were recorded during the month of January at the top of the Kut barrier, reaching 51.20, 15.53 and 8.98 U/L for AST, ALP and ALT enzymes, respectively, while the values increased and recorded the highest rates during August 2021 below the Kut barrier and were 410, 47.52 and 61.12 U/L. for the same enzymes respectively. The present results indicate a decrease in liver enzymes in the blood of fish before the barrier, compared to the fish of the same species below the barrier (Table 4). As we notice a decrease in enzyme activity during the cold months in the first site before the Kut barrier compared to its increase during the hot months at the second site after the Kut barrier, this may be the result of the stress caused by heavy metal pollution in the second site. The increase in water temperature leads to an increase in the enzyme activities due to the increase in the activity of feeding, growth and movement, and thus this leads to an increase in the level of concentrations of these enzymes in general (36).

Table 4. Mean values for the activity of some liver enzymes for Gattan fish caught at the Kut barrier in the Tigris River for the period from January to September 2021

Months	Aspartate Amino Transaminase (AST) U/L		Alanine Transaminase (ALT) U/L		Alkaline Phosphatase (ALP) U/L	
	S1	S2	S1	S2	S1	S2
January	51.20Be	185.60Ad	15.53Be	35.05Ae	8.98Bf	22.09Af
February	55.56Be	205.27Ac	17.80 Be	39.85Ae	10.48Be	27.09Af
March	58.27Be	210.50Ac	22.60Bd	40.76Ad	11.49Bd	26.25Af
April	70.59Bd	298.32Ac	22.75 Bd	40.76Ad	12.55Bc	29.76Ae
June	84.50Bd	300.47Ab	24.35 Bc	42.50Ac	12.95Bc	31.35Ad
July	159.83Bb	354.40Ab	30.58Bb	43.81Ab	13.98Bb	61.12Ab
August	185.60Ba	410.00Aa	35.60Ba	47.52Aa	13.98Ba	72.89Aa
September	148.03Bc	330.00Ab	33.36 Bb	43.81Ab	12.95Bc	50.37Ac
Range	51.20-185.60	185.60-410	15.53-35.60	35.05-47.52	8.98-13.98	22.09-72.89
Mean ± S.E	101.6±19.0	286.82±28.2	25.3±2.5	41.7±1.2	12.17±0.6	40.1±6.6

Different capital letters indicate significant differences within the same row locally at the probability level ($P \leq 0.05$). Different lowercase letters indicate significant differences within the same column per month at the probability level ($P \leq 0.05$).

Results of (23) showed that an increase in the concentration of heavy metals leads to a decrease in the activity of some enzymes and their oxidation to certain molecules, or the rupture of cell membranes in the liver tissues, and the leakage of some enzymes from it into the blood, which reduces its concentration in the liver and increases it in the blood. The relationship between Cadmium, Zinc and liver enzymes in fish blood is a direct relationship, and it is one of the evidences that indicate damage to the liver and its cells due to metal poisoning. Moreover, ALT and AST are biomarkers for detecting hepatotoxicity, while ALP indicates damage to the epithelium of the bile duct.

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