Effect of heavy metals on physiological and histological status in liver of common carp *Cyprinus carpio*, reared in cages and wild in the Euphrates River, Babil / Iraq.

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Abstract. The current study was conducted to examine the concentrations of some heavy metals (Fe, Zn, and Cu) in sediments, chemical and physical properties of water (Temperature, pH, Salinity, Total dissolved solid, Dissolved oxygen, Biological oxygen demands, Alanin transporter enzyme, aspartase transporter enzyme, Alkaline phosphatase, and histological effects of the studied metal on liver of 96 cage fish and wild fish 4 fish per site *Cyprinus carpio* (Common carp) in the Euphrates River from October of 2018 until November of 2019. Three sites were selected on the Euphrates River in the middle of Iraq. The first site was Abu Luka, the second site was Al Saddah and the third site was the village of Al-Hussein. The results showed that in the first and second sites, the concentration of heavy metal in sediments and fish liver was greater than in the third site. In general, the cage fish at the first and second sites were higher than the wild fish. It was also noticed that the heavy metal concentration of both fish and sediment took the following order according to the seasons of the year, from the highest concentration of heavy metals to the lowest (summer > spring > autumn > winter). It was found that liver enzymes were directly affected by the presence of high concentrations of heavy Metals. So, both the ALT and AST increased in the first and second sites as compared to the third site. But the ALP value decreased with the increase in the concentration of the studied metal. The results of the histological slicing of the liver in the first and second places revealed a breakdown of the hepatocytes with the creation of gaps between the cells. Several spaces filled with remains of the destructed cells from the visceral tissue of the liver with infiltration of inflammatory cells in the infected area of the fish liver. It is the region's first study that was concerned with the impact of heavy metal levels in the environment on cage fish and their relationship to blood components and liver enzymes.

Keywords: Aquatic ecosystem, Heavy metal toxicity, *Cyprinus carpio*, Liver, Euphrates river, Babil

1. Introduction
The development of modern industrialization and rapid technology is responsible for aquatic ecosystem pollution. Discharged waste from anthropogenic activities into the water environment has an influence on the fauna and flora[1]. Heavy metals naturally exist at low levels in Aquatic ecosystems. These amounts increase from excreting waste from industrial, agricultural and Sewage water. This rise leads to a decrease in water quality and effects on Aquatic creatures in it because, it becomes more permanent in the ecosystem due to the fac that it does not disintegrate [2]. However, whether their rates are higher or lower than the optimal limits, they inflict Physiological damage and Cause death to Aquatic creatures [3]. Sediments constitute the main mineral reserve in the Aquatic ecosystem and contain high heavy metal concentrations. Furthermore, heavy metals induce the generation of ROS, as well as Free Radicals, which leads to oxidative stress. This leads to an imbalance between oxidants, free radicals, and antioxidants. These are considered responsible for the cellular defense mechanism in living organisms towards the development of more antagonists (AST), (ALT) and (ALP) which are released for acute and
chronic liver disorders. These activities are attributed to the enzymes which are the biomarkers of acute liver injury and can also be used as a diagnostic method to determine liver cell necrosis [4].

2. Materials and methods; Description of the Study Area:
The present study was conducted on groups of some floating cages on the Euphrates River. The first group is in Al-Musayib (Abu Luka), located within the coordinates N32°50’22.02” E44°16’17.731” (Figure 1). The second group is in Sadat Al-Hindi: in the Al-Sadah area located within the N32°44’19.169” E44°16’5.19”
In Figure 2, the third group is in the village of Al-Hussein: (Al-Hussein), located within N32°23’20.767” E44°23’34.922” (see Figure 3).

2.1. Sample collection:
Water samples (3 sample per site) were obtained from the stations on monthly basis particularly from the center of the river along the Euphrates River between October 2018 and November 2019. The sediment samples collected from the studied sites were collected from the area next to the cages. They were placed and kept in a refrigerated box until they reached the laboratory. Fishes (96 sample). They were collected during October 2018 and November 2019. Whereas the samples of wild fish (4 fish per site) were obtained using a cast net Fishes. The caught fish were placed in plastic containers of 30 l and filled with river water. Then they were transported to the physiological laboratory at the College of Veterinary Medicine, in Al-Qasim Green University. In the laboratory, blood drawing and tissue extraction (liver) took place as it helps to estimate the heavy metals since it was preserved under high cooling in the laboratory and reduced with formalin at a concentration of 10 %. As for histopathology, the fish that appeared in good health were selected with an average weight of 1900-2000 g. Then, blood was drawn from various groups of fish directly from the heart or the tail artery (4 fish per site) by 10 ml evacuated tube. The blood serum was then extracted in a centrifuge (3000 d / min for 15 minutes) for performing enzyme tests transporting the amine group AST, ALT and ALP.

Some environmental properties of water were field-measured using a multi-meter (Hanna 9811) and included water temperature (c), pH, salinity concentration (mg / l) and T.D.S. O₂ (mg /L). Then, the blood serum was separated in a centrifuge (3000 d / d for 15 minutes).
2.2. **Trace Metals Extraction:**
The process [5] was used to remove the heavy Metals from sediments and digest the aforementioned fish samples using the digestion procedure using a flame-atomic absorption spectrophotometer (FAAS-Model Shimadzu 6300). Germany supplied a cathode lamp for each element, Affiliated to the Central Laboratory - College of Agriculture - University of Basra.

2.3. **Histological Technique:**
The histological technique described by [6] was being used to extract parts of the tissue. This was conducted in Histopathology for postgraduate studies Laboratory of the Poultry and Fish Diseases department / College of Veterinary Medicine, University of Baghdad, using a tissue processor developed by histo-line Laboratories Company.

2.4. **Statistical Analysis**
The experiment was designed according to completely randomized design (CRD) and the use of the ready statistical analysis system in analyzing the effect of the parameters on the studied traits. The significant differences between the averages of the studied traits were tested according to Duncan’s multiple range test at a significant level [7].

3. **Results**

3.1. **Chemical and Physical Factors**

**Temp:** water temperature levels ranged from its lowest (12.33 °C) in the site (3) during the winter season 2019 to the highest (29.167 °C) in the summer season 2019 at a site (1) as seen in the Table 1. The results showed there were significant differences between the seasons of the study at only (p<0.05).

**PH:** Table 1 shows the seasonal changes in pH values during the study period, as the lowest value was (6.066) at site 2 during the summer of 2019. While the highest value was (8.23) at site 1 during the winter season of 2019.

**Salinity:** The lowest salinity value was reported during the summer at site 3 (0.513), while the highest value was (0.845) at a site (1) during the winter of 2019. We note that the major season variations have exceeded winter over the rest of the year, followed by spring, autumn, and summer, respectively, as seen in Table 1.

**TDS:** The value of total dissolved solids ranged between (401 mg/l) as the lowest value during the summer season at a site (3) and (660 mg/l) as the highest value during the winter season at the site (1) as shown in Table 1. Further, there were variations between the sample groups at a relevant level (p < 0.05).

**O₂:** During the study period, the lowest concentration of dissolved oxygen was recorded as (5.6) mg/l on site (1) during the summer season of 2019. While the highest concentration was (8.133) mg/l on site (3) in January 2019, as shown in Table 1. However, there was no observation of any significant difference between the sites in each spring, winter, and autumn season. As for the significant differences between the season.
3.2. Heavy metals in sediment Zinc:
The study results showed that the lowest value of zinc was 34,204 µg/g in the site (3) during the autumn season, with the highest value being 83,307 µg/g in the site (2) during the summer season, as seen in Table 2. In addition, statistical analysis revealed that there were significant differences between the seasons as well as between the study sites (p<0.05). The zinc recorded the lowest value at the third site, which, as previously mentioned, contains a licensed cage farm. However, the highest values were at the second and first sites, although no significant differences were found between them. However, it was noted that there was a small variation in numbers. As for the seasons of the year in which the research was done, the current analysis showed an increase in the value of zinc during the summer season; followed by spring, then winter, and autumn. Table 2 By comparing these concentrations of the current study for each site and all seasons of the year, we note that the first site shows that the zinc value increased in the summer and spring and decreased in the winter, followed by the autumn season, with a significant difference indicated by statistical analysis results below a significant level (p<0.05). No significant differences between the seasons of the year were noticed as for the second site. On the other hand, the third site shows there is a significant difference in the value of zinc, where the summer exceeded the autumn. As for the winter, the difference with the autumn was not important, but the spring did not notice a change from the season Summer and winter for the third site. As shown in Table 2.

**Copper:** The concentration of copper in the sediments ranged between 18.14-47.863µg/g, and the lowest value was recorded in autumn at the site (3), while the highest value was recorded in summer at the site (2), Table 2. And it was observed that there are significant differences between the months and between the study sites (p<0.05) by statistical analysis. additionally, there was no significant difference between summer and spring, and the autumn and winter results came afterward, and no significant difference was found between them. On the other hand, site (2) shows an increase in the value of Cu relative to the rest of the study sites, and it was found that while there were no significant differences for the same site during the various seasons of the year. It was also found that the summer season has outperformed numerically the rest of the seasons of the year and did not show significant differences as compared to the rest of the seasons that were below the significant level (p <0.05). Furthermore, the value of copper element differed in the third site, so the autumn was at the lowest value, and there was no significant difference between the remaining three seasons, Table (2). This was revealed by statistical analysis (p <0.05). **Iron:** The results of the statistical analysis of the iron indicate

### Table 1: Seasonal changes in the chemical and physical properties of water in the study sites Babil

| Location          | Test | Temp (°C) | PH | Salinity‰ | T.D. | S.Bgl/ml | Site 3 (village of Al-Husayn) | Site 2 (Al-Sadah) | Site 1 (Abu Luka) |
|-------------------|------|-----------|----|------------|------|---------|--------------------------------|------------------|------------------|
|                   |      |           |    |            |      |         | Autumn | Summer | Spring | Winter | Autumn | Summer | Spring | Winter | Autumn | Summer | Spring | Winter |
| Site 3            |      |           |    |            |      |         | 18.83b | 28.97a | 20.467b | 12.33c | 17.967b | 27.73a | 20.13b | 14.33c | 18.86b | 29.16a | 20.8b  | 14.667c|
| Site 2            |      |           |    |            |      |         | ±0.16± | 1.15±  | 1.44±   | ±0.69 | ±0.14±  | 0.99±  | 1.06±  | 0.40±  | 0.61±  | 0.60±  | ±1.33± | ±0.72± |
| Site 1            |      |           |    |            |      |         | 7.033d | 6.1e   | 7.3cd   | 7.8abc | 7.33cd | 6.066e | 7.4bc   | ab7.96 | 7.1d   | 6.2e   | 7.3cd | 8.23a |
|                   |      |           |    |            |      |         | ±0.08± | 0.17±  | ±0.15±  | ±0.05 | ±0.17±  | 0.40±  | ±0.15± | ±0.12± | ±0.12± | 0.23±  | ±0.17± | 0.26±  |
|                   |      |           |    |            |      |         | 0.732e | 0.513g | 0.802c  | 0.83ab | 0.743de | 0.537f | bc0.8  | 0.832a | 0.758d | 0.54f  | 0.80bc | ±0.845 |
|                   |      |           |    |            |      |         | ±0.001±| ±0.007±| ±0.005± | ±0.009| ±0.007± | ±0.0053| ±0.002± | ±0.007 | 0.011± | ±0.005 | ±0.001± | ±0.003± |
|                   |      |           |    |            |      |         | 572.2± | 401.33g| 627.167c| 645.5ab| 581.1cd | 419.6f | 632.6bc | 605a  | 592.5d | 427.6f | 632.5bc | 660.1a |
|                   |      |           |    |            |      |         | ±1.48± | 5.6±   | ±3.91±  | 7.75± | ±5.97±  | 7.28±  | 1.69±  | 5.77±  | ±8.77± | ±3.92± | 1.44±  | ±3.03± |
|                   |      |           |    |            |      |         | 7.1cd  | 6.533d | 7.33bc  | 8.13a | 7cd    | 5.966e | 7.22bc | 7.8ab  | 7cd   | 5.6e   | 6.99cd | 7.76ab |
|                   |      |           |    |            |      |         | 0.15±  | 0.08±  | ±0.02±  | ±0.18 | ±0.11±  | ±0.24  | ±0.06± | 0.26±  | ±0.05± | ±0.30± | 0.12±  | ±0.31± |

Means with the same letter are not significantly different in same row *(± Std Error)*

### Table 2: Seasonal changes in the heavy metal contents in sediments

| Element | Location | Autumn | Summer | Spring | Winter |
|---------|----------|--------|--------|--------|--------|
| Zinc    | Site 1   | 34.04  | 37.33  | 37.04  | 35.04  |
|         | Site 2   | 37.33  | 37.04  | 37.00  | 37.00  |
|         | Site 3   | 34.04  | 37.33  | 37.04  | 35.04  |

Means with the same letter are not significantly different in same row *(± Std Error)*
the dominance of the second site during the summer season, where it value was (342.787) µg/g, followed by the spring season and, finally, the winter season and the autumn season, which revealed no significant difference between them (p < 0.05). It had no significant difference from the second site as with the first site, but the summer season was at the highest value, followed by (spring, winter, and autumn) respectively. The third site had the lowest value in the autumn season when it reached (241.42) µg/g and increased significantly in the winter season where it was (254.167) µg/g, and the highest value came in the summer and spring, with no significant difference between them (269.7), 262.8 µg/g, respectively (p < 0.05). Table 2

| Site 3 (village of Al-Husayn) | Site 2 (Al-Sadah) | Site 1 (Abu Luka) |
|-------------------------------|------------------|------------------|
| **Autumn**                    | **Summer**       | **Spring**       | **Winter** |
| 34.204 ± 2.1                  | 41.69 ± 1.657    | 37.549 ± 1.3987  | 38.368 ± 1.145 |
| 18.14 ± 0.95 ± 0.886         | 22.267 ± 1.037   | 20.818 ± 1.082   | 18.86 ± 1.24 |
| 241.42 ± 2.35 ± 1.12         | 269.7 ± 1.0985   | 262.8 ± 2.8      | 254.17 ± 1.1 |

Means with the same letter are not significantly different in same row *

3.3. Heavy metals in Fish liver

**Zn in the Fish liver:** Table 3 shows zinc concentrations µg/g dry weight in the liver of wild fish and cage fish from the three study sites on the Euphrates River during the study period. Statistical analysis showed that there were significant differences (between the three study sites and between the cage and wild fish, as well as between the seasons of the year) under a significant level (p<0.05). The highest value for cage fish at the second site in the summer 83,307 µg/g and the lowest value for the zinc element of the fish livers was 12,024 µg/g in the winter when cage fish was at site 3. For the summer season, the concentration of zinc in the liver was higher in the fish raised in cages of the second site, followed by the fish raised in cages of the first site and the wild fish of the second site, respectively (hunting the first site, hunting the third site and fish raised in site cages 3). As for the spring season, the highest value was also for cage fish in the second site, followed by the first site, and wild fish from the second site, and then respectively (wild fish in site 1, wild fish in site 3 and fish cages at site 3). The results of the winter season demonstrated that zinc was the highest value of cage fish in the second site and cage fish in the first site with no significant difference between them, followed by wild fish in the second site, then wild fish in the first site, and finally the third site came in, and wild fish exceeded the cage fish. **Cu in the Fish liver:** Table 3 shows the values of dry weight concentrations of copper µg/g for fish’s liver of wild fish and fish raised in cages from the three study sites during the study period. Thus, the importance of copper in the liver for fish raised in cages was higher than wild fish, in first and second sites. The highest value of copper was in second site cage fish in summer, reaching (8.793), followed by cages for the same season at site 1 and then wild fish at both of the first and second sites. Over the summer, it was not noticed that there is a significant difference between wild fish and cage fish at all sites. In the current study, the third site was the lowest value cage fish at the third site was (2.0739 g / g) in autumn. summer season arrangement was as follows: cage fish: site 2 > cage fish site 1 > wild fish at each of the first and second sites > third site wild fish > third site cage fish **Fe in the Fish liver:** Table 3 shows the rates of dry weight iron concentrations µg/g of the wild fish and cage fish livers from the three study sites on the Euphrates River during the study period. So, winter and autumn results were lower than summer and spring results. Accordingly, results showed that the highest value in the summer was 318.181 µg/g for
cage fish at the second location and the lowest value was 192,484 µg/g for cage fish at the third location during autumn and the values were arranged as follows the first and second sites for the summer, followed by cage fish for spring on both of the first and second sites, wild fish for both site first, second and wild fish at the third site, followed by cage fish at the same site.

Table 3: Seasonal changes in the concentrations of heavy metal in liver of *Cyprinus carpio* for the studied areas µg/g

|       | Site 1 (Abu Luka) | Site 2 (Al-Sadah) | Site 3 (village of Al-Husayn) |
|-------|-----------------|-----------------|-----------------|
|       | Winter          | Spring          | Summer          | Winter          | Spring          | Summer          |
| Zn    |                 |                 |                 |                 |                 |                 |
| Cage  | 64.41±          | 78.97±          | 87.49±          | 66.28±          | 76.49±          | 83.206±         |
|       | EF              | EF              | CB              | EF              | CB              | A               |
|       | 2.272±          | 1.308±          | 1.60±           | 1.06±           | 1.504±          | 0.834±          |
|       | ±2.89           | ±1.20           | ±1.11           | ±1.699±         | ±1.756±         | ±2.135          |
|       | F               | B               | FG              | E               | GH              | A               |
|       | 51.435±         | 60.349±         | 58.06±          | 56.17±          | 68.29±          | 74.361±         |
|       | FG              | E               | GH              | GH              | DE              | CB              |
|       | ±2.16           | ±1.48           | ±1.46           | ±1.756±         | ±1.46±          | ±2.135          |
| Cu    | 6.192±          | 7.989±          | 8.289±          | 6.0027±         | 8.23±           | 8.7093±         |
|       | F               | B               | AB              | F               | A               | EF              |
|       | 4.337±          | 7.176±          | 7.884±          | 3.83±           | 6.93±           | 7.77±           |
|       | G               | B               | G               | HI              | DE              | BC              |
|       | ±0.198±         | ±0.07±          | ±0.104±         | ±0.410±         | ±0.020±         | ±0.296±         |
|       | ±4.656±         | ±3.11±          | ±3.96±          | ±1.118±         | ±0.808±         | ±0.066±         |
|       | ±0.126±         | ±0.286±         | ±1.126±         | ±0.080±         | ±0.296±         | ±0.126±         |
| Fe    | 0.327±          | 0.173±          | 0.092±          | 0.410±          | 0.16±           | 0.219±          |
|       | CD              | B               | A               | HI              | DE              | BC              |
|       | 3.023±          | 3.11±           | 1.483±          | 1.575±          | 4.69±           | 9.09±           |
|       | ±2.848±         | ±1.245±         | ±0.879±         | ±2.679±         | ±4.657±         | ±3.121±         |
|       | ±2.90±          | ±1.16           | ±2.90±          | ±1.16           | ±2.90±          | ±2.90±          |

Means with the same letter are not significantly different in same row*±* Std Error *

3.4. Alanin transporter enzyme ALT or GPT

There are no significant differences between the seasons of the year in the third site. The highest value (8.023) recorded in the autumn season for second site wild fish was as shown in Figure 4. Whereas the lowest value was for the third site wild fish in the winter season (3.71). It was noticed that the first and the second sites for all seasons outperformed the third site (P <0.05). In the first location, the cage fish except in spring season (5.51) and the wild fish of the same site did not show any significant difference between the seasons of the year for the cage fish in the second site except for the winter season that recorded the lowest value of (4.78) at a significant level (P <0.05).

3.4.1. Aspartase transporter enzyme AST or GOT: The results showed that the highest value in the first site’s cage fish for spring season was 120.397 whereas, the lowest value was a record in the third site wild fish of winter season 46,592. The statistical analysis revealed that cage fish in the first location had the highest value for the spring season, followed by the autumn and winter seasons, which had no significant difference. Unlike the other season, the summer season recorded the lowest value (81.84). While wild fish in the first site showed no difference between spring and autumn, then the summer and winter, which also did not have a significant difference. In addition, the second site for cage fish showed the highest value for it was in spring (110.955) then it was followed by winter, autumn, and finally summer that comes with a value of (77.45). On the other hand, the wild fish of the same site showed no differences between them except in the autumn season, which came with the highest value (94,149). It was also noticed that there was no significant difference between seasons in the third site’s cage fish.
except in the spring season, which outperformed the rest of the year with a value of 62,035. Wild fish were at the highest values for the summer and autumn seasons, which had no significant difference between them, followed by winter and spring season, which also did not show a significant difference between them, (see Figure 5).

Figure 4: Seasonal changes in ALT u/l

Figure 5: Seasonal change in AST u/l

3.4.2. Alkaline phosphatase ALP. The results of the statistical analysis showed significant differences between the three sites of the study. So, the highest value was in the third site, followed by the first and the second sites, the highest value was the record of wild fish in winter (106.05), followed by cage fish in the third site in the winter season (105.838). The lowest value was for cages fish of the first site in the autumn season, with a value of (65.793). The results were taken according to the locations for the seasons of the year as follows: The first location of the cage fish, the following ordering pattern: Summer> Spring> Winter> Autumn. For wild fish in the same site, no significant difference was noticed between the seasons of the year except between the autumn and the spring season. As for the second site, the results of the statistical analysis indicated that the cage fish were in the following order: Summer> Spring> Winter> Autumn while there was no significant difference between the seasons of the year of wild fish in the second site when winter and summer seasons were higher than spring and autumn in the cage fish in the third site, and wild fish also came with a higher winter and summer than spring and autumn (see Figure 6).

3.4.3. Histological liver changes. the macroscopic examination doesn’t show any macroscopic pathological modifications of liver size, color, and homogeneity in livers of the common carp at the third site without the existence of any significant macroscopic pathologic lesions (see Figure 7). Histopathological examinations of the livers by microscopy showed the first and second sites of hepatocytes forming (hepatocytes forming) with the formation of spaces between cells. Some spaces filled with remains of broken cells (necrotic cell debris) from the liver parenchyma with inflammatory
cell infiltration in the fish liver affected area (Figure 8), where the image (A) is for the liver of wild fish, and image (B) is for the liver of cage fish, where the symptoms appeared in each of them.

![Figure 6: seasonal change in ALP u/l](image)

**Figure 6:** seasonal change in ALP u/l

![Figure 7: Photomicrograph of common carp normal liver histology x200. H& E.](image)

**Figure 7:** Photomicrograph of common carp normal liver histology x200. H& E.

![Figure 8: photomicrograph of common carp liver histology. A& B/ the necrosis of hepatocytes forming a space (black arrows) some filled with necrotic cells debris, which is observed in liver parenchyma, also infiltration of inflammatory cells (yellow arrows) was noted in affected areas. A& B: x400. H& E.](image)

**Figure 8:** photomicrograph of common carp liver histology. A& B/ the necrosis of hepatocytes forming a space (black arrows) some filled with necrotic cells debris, which is observed in liver parenchyma, also infiltration of inflammatory cells (yellow arrows) was noted in affected areas. A& B: x400. H& E.

4. Discussion

**Temperature:** These results are in agreement with many studies conducted on the Euphrates River in central Iraq, including [8]. It was found that there is a direct relationship between temperature and heavy metal, as shown in Table 1. The reason for this might be attributed to the temp that has an important effect on the chemical reactions of the aquatic environment. This is because it can directly affect on metabolic activities of organisms. In addition, that this was consistent with several studies on the Euphrates River in the city of Babil, including [9][10].

**pH:** The reason for the differences in the pH value during the seasons of the year may be due to the abundance of aquatic plants and phytoplankton, which leads to an increase in the effectiveness of photosynthesis. Then this leads to the consumption of carbon dioxide in the water and raises the pH values and this has been confirmed by several studies([10, 11]). The results of the current study showed that the pH value ranged between (6.66-8.23) throughout the study period in all Locations The water of the Euphrates River tends to be light alkaline, and the narrow ranges of pH values are due to the Euphrates having a buffer capacity that resists changes in pH values.

**Salinity:** In the current study, the water of the river (the Euphrates River) was classified as oligohaline, as its rates ranged between (0.513-0.845). The results show that salinity values increased in the second
site due to the large flows of industrial and agricultural wastes to the river. The results of the current study showed an increase in salinity values during autumn and winter seasons. This may be due to the increase in the entry of dissolved ions by washing soil with rainwater into the river. These results are in agreement with several local studies on the Euphrates, including ([12][13]).

**Total dissolved Solid (TDS):** The high values of total dissolved solids in the winter season are attributed to the rain, especially in densely populated cities and industrial areas. In the same context mentioned [14] the reason for dissolved solids increase when the amount of water in the stream increases. Furthermore, due to the increase in contact with rocks, he mentioned that there is a relationship between salinity and the concentration of dissolved solids, as the higher values of the dissolved solids recorded correspond to the higher values of salinity.

**O₂:** The reason for the high values of dissolved oxygen in the winter is due to the decrease in temperature, which leads to an increase in the solubility of oxygen, and this is confirmed by the correlation between oxygen and temperature, while the decrease in the concentration of dissolved oxygen in the summer may be attributed to the high temperature and the lack of operation Photosynthesis in addition to the river’s effect on the excreta of the river and that the process of its decomposition requires the consumption of dissolved oxygen. This is confirmed by the correlation between oxygen and the vital oxygen requirement. The results of the study agreed with the results obtained by [15]. *Heavy metals in sediment:* It was observed during the results of the study that the first and second stations recorded the highest levels of heavy metals studied and appeared in the following order: Iron> zinc> Copper because these stations are located near the thermal power plant and the concrete factory is exposed to industrial pollutants and the movement of boats used for fishing. These factors add a high percentage of pollutants to the rivers. This percentage was in agreement with [16]. The results indicated that the third station was the least with the values of the heavy Metals studied and the reason may be due to That is to its distance from the sources of pollution and factory waste. In the same context, [17] found the high concentration of heavy metal in sediments compared to the tissues of the studied fish. Regulating the absorption and consumption of heavy metal and indicating that a high concentration of iron and zinc in fish tissues is due to the high concentration of these minerals in the sediments. Thus, these results are in agreement with international studies, including [18]. River in the study area over the course of a year, where the highest concentration of heavy metals studied was more in the summer and spring seasons than in the winter and autumn. On the other hand, the results of the current study agree with several studies conducted on the Euphrates, including[19, 20], where they mentioned the high concentration of Heavy Metals in the spring and their decrease in the winter. The reason for this is that the high temperature works on Increased evaporation of water from the river, and thus the concentration of Metals increases. The seasonal fluctuations of heavy metals in the ecosystem are affected by some of the physical characteristics of the water such as temperature and salinity as well as the chemical properties of the water including pH and dissolved oxygen levels [21].

**Heavy metals in Fish:** The results showed that the accumulation in the liver differed according to the mineral studied, the season of the year. The results of the showed an increase in the value of Heavy Metals in the summer, followed by spring, then autumn, and winter, and the reason for this may be due to the increase in water temperature in the summer, which has a positive effect on the biological activity of freshwater fish, which led to an increase in metabolic activities. For fish in this season, increased intake and accumulation of heavy metals in the tissues. These results are consistent with what he found [19], and he explained that higher temperatures increase metabolic activities and fish appetite, and international studies have also noted that including [18] were indicated that the highest value of heavy Metals is in the summer and the lowest value of the concentration of heavy metal is in the winter, explaining this as above. While the results of the current study agreed with [22], as it was reported that the highest concentration of heavy metal was in the summer and spring in a study conducted Or, the reason may be that the temperature changes the activity of the enzymes responsible for this process and affects the rate of fish absorption of Heavy Metals. The liver is the central active site for absorption and storage of minerals, and plays an important role in detoxification, excretion, and excretion of heavy metals, and it is the target organ for mineral absorption and is considered an indicator of water pollution in aquatic environments. ([23]. These results

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**Table: Total dissolved Solid (TDS):**

| Season   | Concentration of Total dissolved Solid (TDS) |
|----------|---------------------------------------------|
| Winter   | High values of total dissolved solids       |
| Autumn   | Low values of total dissolved solids        |
| Spring   | Intermediate values of total dissolved solids |
| Summer   | High values of total dissolved solids       |

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**Table: Heavy metals in sediment:**

| Mineral  | Concentration in Sediment |
|----------|---------------------------|
| Iron     | Highest concentration     |
| Zinc     | Second highest            |
| Copper   | Lowest concentration      |

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**Table: Heavy metals in Fish:**

| Mineral  | Concentration in Fish Tissues |
|----------|------------------------------|
| Iron     | Highest concentration        |
| Zinc     | Second highest               |
| Copper   | Lowest concentration         |
agreed with what was found that the iron mineral concentration in the liver tissue of all the studied fish was the highest compared to the concentration of other minerals, and the reason for this phenomenon may be that iron is one of the main or important components in the liver tissue. Several studies reported that the accumulation of high iron in the liver [18] as for zinc, it was higher than copper in the liver of fish. The reason for this is the importance of this metal in living organisms that live in the aquatic environment. This is also confirmed by ([24] and mentioned the same previous reason. copper was the least concentrated metal in the liver of fish. This may be because copper is an essential element necessary for the manufacture of hemoglobin, through its entry into some glycoprotein enzymes involved in the production of melanin and catecholamine, which is responsible for the absorption and transportation of iron by converting it into ferric to ferrous, as well as in detoxification processes [18].

**fish blood serum:** The results of the study showed an increase in the activity of the liver enzymes ALT and AST in fish blood serum samples during the study period in the first and second stations, which contain higher concentrations of heavy metals compared with the third area. The reason may be due to the sensitivity of these enzymes to the concentrations of iron, zinc, and copper in the liver of fish, as they respond in different ways to heavy metals in the tissues and organs of fish that are exposed to heavy metal contamination in their environment in which they live in the field and laboratory experiments [25]. The lack of response of some enzymes in the low level of concentration of these minerals may be due to less than the level to which these enzymes respond, due to the function of the liver in dealing with excess and harmful substances and ridding the body of them, and accordingly, the enzymes respond to heavy metals either with increase or decrease. An elevated ALT usually indicates damage to the liver or deterioration of its cells, and this disorder may occur in other organs that secrete this enzyme, such as the heart and muscles [26]. As for the elevation of the AST enzyme, it indicates the tissue’s need for energy as a result of stress and tissue weakness because this enzyme is a key intermediary in the Krebs Cycle [27] and the reasoning behind this is that toxic substances lead to disruption. In the physiological state of the animal because it causes abnormalities in the cell organelles, which can result in an increase or inhibition in the activity of various enzymes. In the same context, the study of [28] that increasing the concentration of heavy metals leads to a decrease in the activity of some enzymes, and this effect leads to oxidation of specific molecules in these enzymes or perhaps due to some heavy metals that leads to the rupture of cell membranes in the liver tissue and this, in turn, causes some enzymes to leak from the liver tissue into the blood, which reduces its concentration in the liver and rises in the blood. The results of the current study showed that the value of (ALP) in the blood of fishes in the first and second site was less than that of the third site. In the same context [29], the correlation between minerals (Cd and Zn) and liver enzymes ALT and AST (increased post-exposure) was confirmed, and the reason for this was due to increased levels of liver enzymes in the blood, which is the main indicator that informs us about liver and cell damage. Caused by metal poisoning. In addition, the enzymes ALT and AST are used as biomarkers to detect hepatotoxicity, while ALP indicates damage to the epithelium in the bile duct [29]. The increase in liver enzymes may be attributed to a result of the impairment of liver tissue cells and the release of enzymes into the blood and are used as indicators of stress, as well as the result of severe damage to the liver tissue due to heavy metals. These enzymes are used as an indicator of liver susceptibility to toxic substances [30].

**Histological Changes for liver:** The results of histological examination of fish liver tissue showed the distraction of hepatocytes and the formation of spaces between hepatic cells, and some spaces were filled with the remains of the destroyed cells from the parenchyma of the liver with infiltration of inflammatory cells in the affected area. The results are in agreement with [31] who reported that exposure to a chemical such as heavy Metals causes vacuolization in the liver tissue. In the same context, [32] stated that the formation of vacuolization between hepatocytes was the most pathological sign in fish exposed to pollutants, especially heavy Metals. [33] reported congestion in sinusoids and blood vessels in fish collected from sites contaminated with toxic substances. In general, the liver shows reliable results for EQA [34], the histological changes detected here in the livers of fish from all investigated regions confirmed that the fish were affected by the high level of heavy metals detected in the environment and liver tissue.
5. Conclusions
The influence of environmental factors on the prevalence and accumulation of heavy metal inside the fish liver and sediments. The presence of seasonal variation in the concentrations of the studied metals in the sediments of the Euphrates River, as they were high in summer and low in winter. The accumulation of heavy Metals in polluted areas is higher in the livers of cage fish than in wild fish, and in areas free from contamination with heavy metal, the accumulation of heavy metals in wild fish is higher than in cage fish liver enzymes have different responses to the concentrations of heavy metal, which either increase or decrease depending on the type of enzyme and the physiological response of the fish.

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