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Histopathological Effects of Some Heavy Metals and Environmental Factors on Brain of Common Carp *Cyprinus carpio,* Reared in cages and Wild Fish in Euphrates River, Babil, Iraq

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Abstract: The current study was conducted to study the concentrations of some heavy metals (iron, zinc, and copper) in sediment, water temperature, pH, salinity, T.D.S,O_2,bod5, and histological effects on the brain of cage fish and wild fish Cyprinus carpioin the Euphrates River from October of 2018 until November of 2019. Three sites were selected on the Euphrates River in the middle of Iraq first site was Abu Luka in Musayyib near the thermal power station second site was Al Saddah near the cement factory third site was in the village of Al-Husayn. Results showed that in the first and second sites the concentration of heavy metal in sediments and fish brains was greater than in the third site, In general, cage fish at the first and second sites were higher than wild fish with their contents with heavy metal because they were held close to the sources of pollution. It was also noticed that the heavy metal concentration of both fish and sediment takes the following order according to the seasons of the year (summer > spring > autumn > winter), so they have a positive correlation with both the temperature and bod5 and inversely correlation with the pH, salinity and T.D.S and O2, The results of histological examination of the brain showed cellular degenerative changes in telencephalon brain and metencephalon brain tissue. The pathological damage was characterized by the presence of vacuolation between neurons in regions of the telencephalon brain and metencephalon brain near the stratum per ventricular layer with the presence of inflammatory cell infiltration and presence necrosis of neurons and glial cells in visceral tissue. The concentration of zinc was below the international determinants of World Health Organization and Canadian guidelines as well as the Environmental Protection Agency. But was higher than Food and Agriculture Organization Standards. The value of copper was higher than the World Health Organization's international specifications, the Food and Agriculture Organization, and Canadian specifications, but it is within the limits allowed by the US Protection Agency.

Keywords: *Cyprinus carpio*, Babil, Heavy metal ecological, Histopathology, Fish Cages, Euphrates River, Iraq

Heavy metal pollution is a source of great concern due to its harmful effects on the environment, animals, and human health (Yarur et al 2019). In the past few decades, heavy metals have been polluted in aquatic environments in abundance due to increasing urbanization (Carolin et al 2017). Heavy elements are metallic elements that have a relatively high density and are toxic even if they are in low concentrations in the bodies of living organisms. (Tiimub et al 2013). (Díaz et al 2018) mention Aquatic ecosystems are more sensitive to heavy metals than terrestrial ecosystems. Heavy metals are non-degradable and can easily accumulate in living organisms, including fish (Strungaru et al 2018)There are several ways to accumulate heavy metals in fish through direct absorption from water and food (Shesterin 2010). Each of the heavy elements has physical properties, so it accumulates in certain parts of the fish body tissues more than the rest of the other body tissues (Jon et al 2006, Uysal et al 2008)

Moreover, heavy metals entering fish through the gills have a chance to accumulate in various body systems and excessive amounts of them can reach a level toxic to humans (Kumar et al 2007), can cause hepatotoxicity, or To the kidneys (nephrotoxicity), to the central nervous system (neurotoxicity), or to the genes (genotoxicity) (Sharma et al 2014, Gupta et al 2015).

It was mentioned by (Su et al 2013) that histological biomarkers are used consistently in most toxicological studies because they indicate the general health of fish and are important indicators of environmental pollution (Omar et al 2013). As the concentrations of heavy metals in the muscles are often used as an indicator for assessing the pollution of the aquatic environment in the long term and evidence of the health risks of fish consumption by humans (Islam et al 2015).

Babil Governorate is considered a center for fish farming in Iraq and due to the presence of many industrial facilities that dump their waste untreated into the Euphrates River, which leads to increased levels of pollution and negative damage to fish. Therefore, the study aims to Measure some of the physical, chemical, and physiological environmental factors of the specific sites on the Euphrates River, measuring the concentration of heavy metals in the sediments of the studied sites on the Euphrates River. Studying the histological changes in the brain of fishes from floating cages and the river from which they were caught due to the presence of pollutants.

MATERIAL AND METHODS

Description of the Study Area: The present study was conducted on groups of some floating cages on the Euphrates River in Hillafirst group in Al-Musayyib Floating cages in the Abu Luka region, north of Hilla and located within the coordinates N32˚50'22.02" E44˚16'17.731"It consists of 40 floating cages with dimensions 4x4x2 meters for each cage, the distance between the base of the cage and the river bed 2-3 meters depending on the distance or location of the cage from the beach. The river is about 110 m wide and 6 m deep. The density of agriculture is 78 fish/m^3 or around 2500 fish per cage (Fig. 1). The second group in Sadat Al-Hindi Floating cages in the Al-Sadah area, north of the city of Hilla and is located within the coordinates N32 ˚ 44'19.169" E44 ˚16'5.19"It consists of 70 floating cages with dimensions 4x4x2 meters for each cage, the distance between the base of the cage and the riverbed 2-3 meters depending on the distance or location of the cage from the beach. The river is about 100 m wide and 6 m deep. The density of agriculture is 75- 50 fish/m^3 or around 2500 - 1500 fish per cage, and floating feed is used to feed the fish. The third group in the village of Al-Husayn Floating cages located in the village of Al-Husayn, south of the city of Hilla, located within the coordinates N32˚23'20.767" E44˚23'34.922 "It consists of 20 floating cages with dimensions 3x3x2 meters for each cage, They are floating cages that represent a control station because they are located relatively far from the sources of industrial pollution and on another branch of the river, That they perform all Ministry of Agriculture and Environment requirements. The depth between the base of the cage and the bottom of the river is 3 meters. The width is 65 m and the depth are 10 m. The culture density is 44 fish / m3 and the floating feed is used for feeding fish.

Sample collection: Water samples were obtained from the stations on a monthly basis from the center of the river in October 2018 and November 2019 Sediment samples from the sites studied were collected from the area next to the cages, They were placed and kept in a refrigerated box until they reached the laboratory, then held in the freezer until the measurements had been made.

Samples of wild fish were obtained using a cast net. The caught fish were placed in plastic containers of 30 liters and filled with river water. For a period of three hours, they were transported under refrigeration to the physiological laboratory at the College of Veterinary Medicine, in Al-Qasim green University, tissue extraction (brain) For the purpose of estimating heavy metals. Since it was preserved under high cooling in the laboratory and reduced with formalin at a concentration of 10 % for histopathology, fish that appeared in good health were selected with an average weight of 1900-2000 g. Some environmental properties of water were field-measured using a multi-meter and included water temperature ©, pH, salinity (mg /l), and T.D.S. O2 (mg / L) The BOD5 procedure described in (Rice et al 2012) was used to approximate the bio-requirement for oxygen by calculating the (primary) dissolved oxygen in the water sample. The samples were incubated for 5 days at a temperature of 20 0 C with dark Winkler bottles in a dark place. From the following equation, then extract the essential oxygen requirement:

BOD5 = Amount of dissolved oxygen (primary) – amount of final dissolved oxygen (after incubation)

Trace metals extraction: After removing the solid parts from them, the sediment samples were dried at 80°C for 48 hours, milled using a Swiss-made Blender model electric mill, and then passed through a 0.4-micron-sieve to get minutes of less than 63 micros. The process (Moopam 1983) was used to remove heavy metals from sediments and digest the aforementioned fish samples using the digestion procedure until a flame-atomic absorption spectrophotometer equipped with a cathode lamp for each element, Affiliated to the Central Laboratory, College of Agriculture, University of Basra.

Histological technique: Samples of the brain were obtained, and then the histological technique described by (Luna 1968) was being used to extract parts of the tissue. Which 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.

Statistical analysis: The experiment was designed according to the completely randomized design (CRD) and were analysed to Duncan's multiple range tests.

RESULTS AND DISCUSSION

Chemical and Physical Factors

Temp: water temperature levels ranged from its lowest (12.33°C) in site (3) during the winter season of 2019 to the highest (29.167°C) in the summer season of 2019 at site (1) and as seen in the table (1). The results showed there were significant differences between the seasons of the study ($p<0.05$), No major variations in water temperature between sites have been observed ($p<0.05$). These results are in agreement with many studies conducted on the Euphrates River in central of Iraq, including Jubouri (Al-Jubouri 2019). In the current study, and through statistical analyzes, it was found that there is a direct relationship between temperature and heavy metal, as shown in Table 1. The reason for this may be that the temp has an important effect on the chemical reactions of the aquatic environment because it has the ability to directly effect on metabolic activities of organisms and that this was consistent with several studies on the Euphrates River in the city of Hilla, including (Hammoud et al 2017) and (Samer Saleem Alshkarchy Amjed K. Raesen 2020), where they also indicated that there is a direct correlation between temperature and the concentration of heavy metal

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 and the highest value was (8,23) at site 1 during the winter season of 2019. The statistical analysis showed substantial variations across the seasons of study ($p < 0.05$). 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 and then leads to the consumption of carbon dioxide in the water and raises the pH values and this has been confirmed by several studies. Local ones (Kassim et al 1997, Abowei 2010). Or perhaps the cause of this dilution in the water as a result of increased precipitation and washing the soils adjacent to the river (Al-Mousawi et al 1994). 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 and this has been confirmed by several studies, including (Hassan et al 2010).

Salinity: The lowest salinity value was reported during the summer at site 3 (0.513), while the highest value was (0.845) at the site (1) during the 2019 winter. The results showed that during each fall season there were significant differences between the study sites, where the first site was higher than the site (3). The first and second sites were greatly superior to the third site for the summer season. It was observed in the same way, by the winter season's statistical analysis. On the other hand, we note that the major seasonal variations have exceeded winter over the rest of the year, followed by spring, autumn, and summer, respectively, as seen in Table 1. It was observed from the results that salinity values increased as we went south towards 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 the fall and winter seasons, and 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 (Bernet et al 2001, Hassan et al 2010) or perhaps the high salinity values are attributed to the mixing processes and the rise of materials from the deep layers to the surface layers. This is what was indicated by (Al-Fatlawi 2005) in a study he conducted on the Euphrates River. Water too. In addition to that, the solubility of heavy metal in the water increases as the salinity values decrease (Phillips et al 1978).

TDS: The value of total dissolved solids ranged between (401 mg/liter) the lowest value during the summer season at site (3) and (660 mg/liter) the highest value during winter season at site (1) as showed in table (1). Statistical analyses showed that there were variations between the sample groups at a relevant level ($p < 0.05$). The highest values of dissolved solids were observed in the winter season, and no significant differences were noted within the study sites, followed by spring and autumn and then summer, as it seemed that the lowest value in all of the above seasons was the third site where there was a significant difference from the first and second sites; which did not show a significant difference Between them The high values of total dissolved

Table 1. Seasonal changes in the chemical and physical properties of water in the study sites

Location Test	Site 1 (Abu Luka)				Site 2 (Al-Sadah)				Site 3 (village of Al-Husayn)			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Temp c	14.667c	20.8b	29.167a	18.867b	14.33c	20.133b	27.733a	17.967b	12.33c	20.467b	28.967a	18.833b
PH	8.23a	7.3cd	6.2e	7.1d	7.96ab	7.4bcd	6.066e	7.33cd	7.8abc	7.3cd	6.1e	7.033d
Salinity ‰	0.845a	0.8096bc	0.540f	0.758d	0.832a	0.8098bc	0.537f	0.743de	0.826ab	0.802c	0.513g	0.732e
T.D.S mg/l	660.167a	632.5bc	427.667f	592.5d	650a	632.667bc	419.667f	581.167ed	645.5ab	627.167c	401.33g	572.167e
O_2 mg/l	7.7667ab	6.993cd	5.6e	7cd	7.803ab	7.22bc	5.966e	7cd	8.133a	7.33bc	6.533d	7.1cd
BOD_5 mg/l	1.286ef	2.2667c	4.42a	2.45c	1.2ef	2.1667c	4.16a	2.41c	0.8033f	1.5433de	3.0967b	1.8833cd

solid in the winter season are attributed to the rains when they fall, especially in densely populated cities and industrial areas. In a study, it carried out on the Shatt al-Hilla, where he mentioned that dissolved solids are directly proportional to the temperature, and in the same context he mentioned (Jawad 2012), in an environmental study he conducted on the Euphrates River explained the reason of dissolved solids increase when the amount of water in the stream increases 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.

Dissolved oxygen O_2: During the study period, the lowest concentration of dissolved oxygen was recorded and was (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. The statistical study showed that the third site in the summer season outperformed the first and second sites, which showed no significant difference between them at a significant level (p<0,05), although it was not observed that there was a significant difference between the sites in each spring, winter, and autumn season. As for the significant differences between the seasons, the results of the statistical analysis confirm that there is a significant difference between them, where the winter, followed by spring and autumn, respectively, is below the significant level of (p <0.05) 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 con 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 (Al-Hilli 2019) in a study he conducted on the Euphrates River. In the city of Samawah to study the structures of the fish community and some environmental and health influences, On the other hand, the lowest value of dissolved oxygen in his study was in the spring, while in the current study it was in the summer The reason for the difference may be due to the different sampling sites and collection time.

BOD5: During the winter season of 2019 at a site (3) the lowest value was registered (0.8033) mg /l and the highest value (4.42) mg/l in the summer season of 2019 at a site (1) in Table 1. The statistical analysis showed that there were significant differences between the semesters of the study, as the summer season was high in the value of bod to the rest of the study seasons, followed by the fall and the spring, which did not indicate a significant difference between the first and second sites. As for the third site, there have been significant differences in all seasons. These results were in agreement with(Al-Rubaie et al 2011)in a study he conducted on the Diyala River to study the effect of organic pollution. On some types of fish and some bottom crustaceans. The values of the BOD in the Euphrates water did not exceed the limits permitted by the international determinants of (3-6) mg / liter, due to the ability of the Euphrates water to self-purify and the great dilution of pollutants due to the large cross-sectional area and continuous good aeration(Al-Sultani 2011, Khudairi 2014).

Heavy Metals in Sediment

Zinc: The study results showed that the lowest value of zinc was 34,204 µg/g in site (3) during the autumn season, with the highest value being 83,307 µg/g in site (2) during the summer season, as seen in Table 2 statistical analysis revealed that there were significant differences between season as well as between the study sites (p<0.05). Where the zinc recorded the lowest value at the third site, which, as previously mentioned, contains a licensed cage farm. The highest value was at the second and first sites, although it was not found that there was a significant difference between them, although it was noted that there was a small variation in numbers between them. As for the seasons of the year in which the research was done, the results were found 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 for 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 for the second site. On the other hand, the third site shows there is a significant difference in the value of the 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 a site (3), while the highest value was recorded in the summer season 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. 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 found that the summer season has outperformed numerically the rest of the seasons of the year and did not show significant differences between it and the rest The seasons of the year are below the significant level ($p <0.05$). 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 the dominance of the second site during the summer season, where its 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.

This percentage was in agreement with (Al-Dehaimi 2010), where it indicated that the main reason for the pollution of the Euphrates River water with heavy metal is due to industrial sources ' disposal of waste without treatment. The results of the statistical analysis indicated that third station was the least with the values of the concentration of 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,(Al-Lahibi et al 2016).(Al-Khafaji et al 2011) found in a study he conducted on the Euphrates River in the city of Nasiriyah to estimate the heavy metal in sediments and common carp, 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 high concentration of iron and zinc in fish tissues is due to the high concentration of these minerals in the sediments. These results are in agreement with international studies, including (Ben Salem et al 2014), which indicated that the concentrations of heavy metals in sediments are higher than those measured in water and fish samples. The reason for this is that the sediments act as a sink for heavy metal metals, or the reason may be due to the tendency of most suspended particles to bind with minerals, forming complexes containing more complex particles and then being deposited in the sediments and gradually accumulating and this is confirmed by (Al-Khafaji et al 2011), where it was mentioned that sediments are the final basin for many plankton in the water column, including heavy metals whose final fate is sediments, so they are naturally present if their source is from nature or by human activities industrial and agricultural waste and sewage.

The results in the current study also revealed a seasonal difference in the concentration of heavy metals in sediments in the Euphrates 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. These results differed with (Al-Khafaji et al 2013), where noted that the lowest value of heavy metal is in the spring and the highest value in the winter season. The reason for this difference between the current study and its study may be due to the location of the study conducted by the current study.

On the other hand, the results of the current study agree with several studies conducted on the Euphrates, including (Salah et al 2012, Farhood 2015) 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

Table 2. Seasonal changes in the concentrations of heavy metal in sediment for the studied areas µg/g

Location/ Element	Site 1 (Abu Luka)				Site 2 (Al-Sadah)				Site 3 (village of Al-Husayn)			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Zn	72.909bc	77.78abc	80.235a	71.773c	77.462abc	83.307a	82.82a	79.836ab	38.36ed	37.54ed	41.69d	34.204e
Cu	35.942e	39.797ed	43.84bc	38.286de	35.773cd	45.353ab	47.863a	41.217e	18.86gf	20.81gf	22.267f	18.14g
Fe	294.053f	314.84cd	333.533ab	300.106ef	306.968def	323.167bc	342.78a	309.01de	254.1h	262.8gh	269.7g	241.42i

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

properties of the water including pH and dissolved oxygen levels (Wong et al 2001). Several international studies also confirmed this reasoning, where (Rajeshkumar et al 2018) stated when noticing the high concentration of heavy metals in summer compared to winter, this could be due to increased water evaporation and less precipitation, which led to an increase in the concentration of heavy metals, and in the same context, an illness (Duman et al 2012). The reason for this is that the heavy metal concentration is lower in winter and autumn compared to spring and summer due to increased monsoon precipitation, which may lead to dilution of heavy metals throughout the rainy seasons.

Heavy Metals in the Fish Brain

Zn in the fish brain: Table 3 shows the rates of zinc concentrations µg / g dry weight of the brain of wild fish and cage fish from the three study sites on the Euphrates River during the study period. It was found through statistical analysis that significant differences exist between the three study sites and between cage fish and wild fish, as well as between Seasons ($p < 0.05$). Cage fish in each of the first and second sites had the highest concentration of zinc component in the brain, ranging between (48.201-33.113) and (50.932-43.294), respectively, compared to wild fish in the same two sites where the values ranged between (36.639-25.465) and (34.309-23.713 respectively). As for the third site, the wild fish at concentrations ranged between (22.319-15.736) values higher than the cage fish in which the zinc ranged from (14.453-10.653). The summer season showed an increase in the value of the zinc component in fish brains with a concentration (of 50.932 In the second location of cage fish, followed by spring, then autumn and winter, respectively, as the lowest value of zinc was reached in the third site for cage fish in the winter season (10.635). Regarding the summer, the highest value of cage fish was in the second site, followed by the first site, and then wild fish in each of the first and second sites, which did not notice a significant difference between them, followed by wild fish from the third site, and then cage fish in the third site. Then came the spring season, the results of which showed that the second site of cages had the highest value in each of the first sites, followed by the second site, after which the wild fish in each of the first and second sites, in which there was no moral difference between them, then the third site was fishing, followed by the third site cages. The highest value recorded in the fall was in the second site for fish, cages cultured in cages, followed by the first site also for fish farming in cages, and then wild fish in each of the first and second sites after the lowest value. The third site was for wild fish, followed by fish farmed in cages for the same site. We note that the winter season took the following arrangement pattern: cage fish in the second site> cage fish in the first site> wild fish in the first site> wild fish in the second site> wild fish in the third site>fish cultured in cages in the third site.

Cu in Fish brain: Table 3 shows the mean concentrations of copper µg/ g dry weight of the brain of wild fish and fish cultured in cages from the three study sites on the Euphrates River during the study period, it was found through statistical analysis that significant differences exist (between the three study sites and between cage fish and wild fish. Also between seasons of the year) ($p < 0.05$). The highest value of copper (7.647 µg / g) in summer was for cage fish in the second site and the lowest value was (1.837 µg / g) for cage fish in the third site. The copper element took the following arrangement pattern in the fall and winter seasons: cage fish for the first and second sites> wild fish in the first and second sites> wild fish in the third site> cage fish in the third site. It is noted from the results of the current study that the highest value of the copper was in the summer and spring seasons, the second site for cage fish, where it was (7.6473 and 7.4687) respectively, followed by cage fish in the second site, then cage fish in the first site, followed by wild fish in each of the first and second sites for both. The seasons, while the third site, wild fish were higher than cage fish, for both seasons (summer and spring)

Fe in fish brin: Table 3 shows the rates of iron concentrations (µg/ g) dry weight of the brain of wild fish and

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

Sit		Site 1 (Abu Luka)				Site 2 (Al-Sadah)				Site 3 (village of Al-Husayn)			
Metal	Fish	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Zn	Wild	25.47fg	30.53e	36.64d	30.68e	23.71fg	31.21e	34.31de	26.46f	15.74ghi	17.42h	22.32g	17.05hi
	Cage	33.11de	42.37c	48.20ab	35.90d	43.29c	48.45ab	50.93a	45.21bc	10.65k	13.22jki	14.45jkh	11.92ik
Cu	Wild	3.78efg	4.52d	4.88d	3.81ef	3.72efg	4.29ed	4.82d	3.73efg	2.65hi	3.35fg	3.79efg	2.55hi
	Cage	5.68c	5.86c	6.74b	5.78c	5.65c	7.47a	7.65a	6.03c	1.84 j	2.57hi	3.06gh	1.99ij
Fe	Wild	266.9fg	280.19de	286.34bcd	268.48fg	265.07fg	275.13ef	288.78bcd	261.83g	216.6j	233.4i	242.86h	214.06j
	Cage	284.2cde	296.46ab	295.39ab	287.15bcd	282.88cde	293.40bc	304.41a	281.15de	167.1k	185.2k	188.46k	166.34k

*Means with the same letter are not significantly different in same Metal cage fish in the three study sites on the Euphrates River during the study period. It was found through statistical analysis that significant differences exist between the three study sites and between cage fish and wild fish as well as Between seasons of the year ($p < 0.05$), the highest value reached 304,409 µg/ g for cage fish in the second site in the summer and the lowest value was 166,341 for cage fish in the third site for the autumn season, where it was found that there is a superiority in the concentration of iron in cage fish in both The first and second sites were followed by the wild fish in both sites. As for the third site, the results were different. The wild fish exceeded the value of the iron accumulated in the brain than the cage fish. You, the results of the comparison between the seasons, were the highest summer values for cage fish in the second and first sites, followed by the wild fish in the first and second sites, and the wild fish in the third site were the highest 242,856 of fish in cages of the same site with a value of 188,456 and the results of the spring season were results of fall were the highest in the value of cage fish in the first site, then cage fish in the second site, followed by wild fish in each of the first and second sites, which had no significant difference between them, and finally the third site in which wild fish surpassed the cage fish. The results of the winter season showed that the cage fish in the first and second sites were of the highest values, followed by the wild fish of the same two sites and finally the wild fish of the third site, and then the cage fish of the same site. The results showed that the accumulation in the brain differed according to the mineral studied, the season of the year and the location of the fish. The reason for this is that differences in the accumulation of heavy metals in fish brain are affected by some factors depending on the metabolism of the element by the fish. The results showed an increase in the value of heavy metals in the summer, followed by spring and then autumn, and winter comes with the lowest value for the concentrations of heavy metals, 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 the metabolic activities of fish. In this season, increased intake and accumulation of heavy metals in the brain. These results are consistent with what was found by (Farhood 2015) in a study he conducted on the Euphrates River near the city of Souk Al-Shuyoukh, where indicated a higher concentration of heavy metals in the summer compared to the winter season, and he explained that higher temperatures increase metabolic activities and fish appetite. An increase in the level of metabolism, which is directly related to the concentration of heavy metals, and international studies also noted that, including (Ben Salem et al 2014) where he indicated that the highest value of heavy metals is in the summer and the lowest value of the concentration of heavy metals in the winter season, explaining this as above. On the other hand (Düşükcan et al 2014) stated in a study to estimate heavy metals in *Luciobarbus xanthopterus* fish that the level of heavy metal accumulation is arranged according to the seasons as follows: Summer> Autumn> Winter> Spring. While the results of this study were as follows: Summer> Spring>Fall> Winter, and the reason for this may be due to the different environmental conditions. 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 results showed that the order of the concentration of heavy metals is iron> zinc> copper. The high concentrations of iron in the brain of the studied fish, which the results showed, as expected, that iron is one of the components of hemoglobin responsible for binding and transporting oxygen in the body. The reason for this may be due to Iron ions precipitate in the form of ferric hydroxide (Fe OH), which is a form of iron that is weakly soluble in water (Tkatcheva et al 2004). Its presence in high concentrations in the environment in which the fish live or to good ventilation, which leads to its mixing in the water. On the other hand (Farhood et al 2020) stated that the concentration of zinc was higher than iron, and the reason for this was attributed to the absorption of the zinc component necessary for metabolism of living organisms in addition to that it works to protect the aquatic environment from the toxic effects of cadmium. The difference from the current study for the difference in the site from which the fish were collected or the different sizes and ages.

Several international studies reported that the accumulation of the high iron component in the organs of them (Öztürk et al 2009, Ben Salem et al 2014) explained the reason for this to the fact that iron is one of the components of hemoglobin responsible for binding and transporting oxygen in the body. It was higher than copper in the brain of fish, and the reason for this was due to the importance of this element in the organisms that live in the aquatic environment, as it is necessary to perpetuate cells, as it enters into the synthesis of several enzymes as an enzyme companion and this is also confirmed (Al-Khafaji et al 2013) and mentioned the same reason Previous and in the same context, (Khudairi 2014)indicated that the zinc component outperformed the copper component per month for the regular carp in its study on the Euphrates River in the city of Hilla.

Copper was the least concentrated in fish brain, and the storage in the tissues of the studied fish was within the limits of homogeneous organization. Perhaps this is due to the fact that copper is an essential element necessary for the hemoglobin, through its entry into some glycoprotein enzymes involved in The production of melanin and catecholamine (which is responsible for the absorption of iron by converting it into ferric to ferrous and transporting it, as well as in detoxification (Ben Salem et al 2014). Or the reason for this may be due, as mentioned (Canli et al 2003) that exposure of fish to heavy metals such as Cu may activate the synthesis of metal-binding proteins that quarantine copper by cadmium and zinc, without producing Toxic effects to fish are considered to be the ability to produce metal-binding proteins (metallothioneins) and their function to bind metals as mechanisms that carry heavy element concentrations (Wagner et al 2003)where metallothioneins belong to a family of low-molecular proteins rich in cysteine involved in the regulation of basic minerals Cu and Zn and eliminating the toxic effects of them (Amiard et al 2006)and this has been confirmed by several local studies, including (Al-Sultani 2011, Khudairi 2014)

Histological changes for the brain: The macroscopic examination showed the absence of macroscopic pathological changes on the brain of common carp fish during the period of study and for all sites in general in terms of size, color, and homogeneity of the brain with no presence of any macroscopic pathological lesions of little importance.

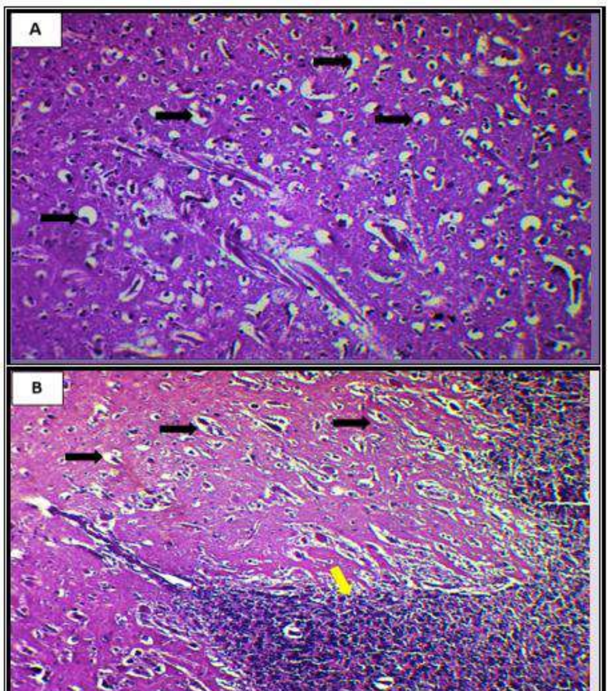

Figure 1: Photomicrograph of brain of common carp fish. A& B: x400. H& E

A& B/ Note the degenerative changes in telencephalon and metencephalon parenchyma of brain. The lesion was characterized by presence of neural vacuolation (black arrows) of telencephalon and metencephalon areas or near the stratum periventricular area (yellow arrow) with infiltration of inflammatory cells.

Results of microscopy of wild fish tissues and the atmosphere of cellular degenerative changes in the telencephalon and metencephalon showed gaps between neurons in regions of the telencephalon and metencephalon near the stratum layer periventricular with infiltration of inflammatory cells and as shown in figure (1), which shows a histological section of the brain of wild common carp during the study period, while cage fish in both the first and second sites showed the presence of neurons cells and cells. Glia in the visceral tissue in the regions of the telencephalon and metencephalon, with gaps between neurons (neural vacuolation), infiltration is also observed in inflammatory cells, as shown in Figure 2, which shows a histological section of the brain of carp fish It is common to be cultured in cages during the study period.

The tissue damage lesion was characterized by the presence of gaps between neurons in telencephalon and the metencephalon near the periventricular layer, with the presence of infiltration of cells and these results were in agreement Al-Bairuty et al (2013) where reported changes in the neuronal bodies of telencephalon and thickening in mesencephalon layers. Bose et al (2013) also reported that the main histological changes observed in the brain of fish

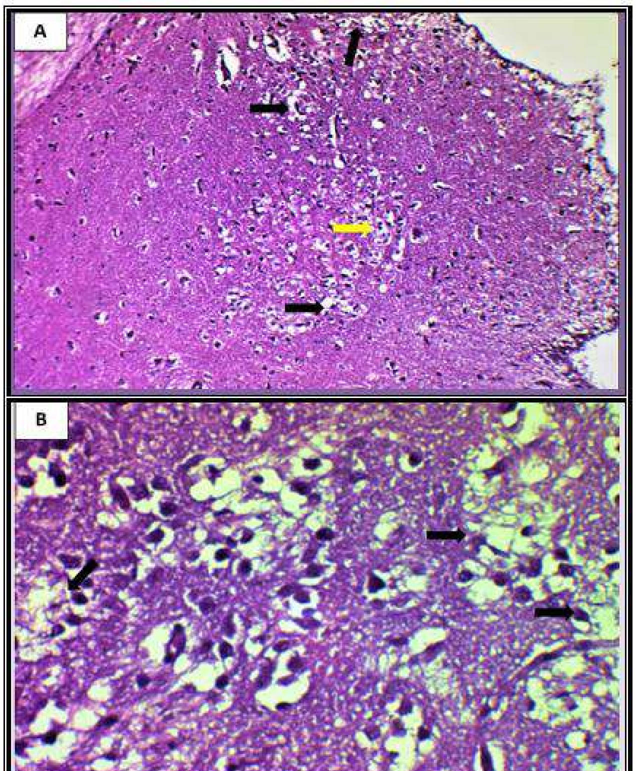

Figure 2: Photomicrograph of the brain of common carp fish. A: x200. H& E. B: x400. H& E.

A &B/ The necrosis of neurons and glia cells were observed in the parenchyma of telencephalon or metencephalon-formed spaces (black arrows), also infiltration of inflammatory cells was observed (yellow arrows).

treated with heavy metals break down into the granular and molecular degeneration of the granular and molecular layer cellular damage in the interior and posterior of brain, degeneration of the nerve cells, vacuolization of the brain cells. The brain has revealed general congestion and meningeal vasodilation along with infiltration of mononuclear cells.

CONCLUSIONS

The presence of an effect of environmental factors on accumulation of heavy metal inside the brain of fish and in sediments, so the H.M had a direct correlation with each of the water temperature and the BOD and an inverse correlation with the pH, salinity, dissolved solids and dissolved oxygen. There is a seasonal variation in the concentrations of the studied metals in sediments. The Euphrates River, as it was high in the summer and low in the winter, and higher concentrations were recorded at Site2, and the lowest concentration of metals was at Site3. The accumulation of heavy metals in polluted areas is in the brain of cage fishes higher from the brain of wild fish and in regions free of heavy metal contamination, the accumulation of heavy metals in the brain of wild fish is higher than the brain of cage fish. The highest element accumulated in the brain of fish and sediments is iron, followed by zinc, then copper.

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