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EFFECT OF POLLUTION OF HEAVY METALS (LEAD, CADMIUM AND MERCURY) ON THE LEVEL OXIDATION OF MALONE DI ALDEHYDE (MDA) IN THE TISSUES CULTURE OF *CYPRINUS CARPIO* IN CAGES ON THE EUPHRATES

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Abstract

This study was conducted during the summer of 2019, and three fish culture sites (*Cyprinus carpio* L.) were selected in cages on the Euphrates within Anbar Governorate, which are under the influence of industrial and human agricultural activities, Where some of the physical and chemical properties of the Euphrates water were measured in the above locations such as temperature, pH, turbidity and total dissolved solids and they were generally within the normal limits, and the level of oxidation was examined (MDA) In the tissues of the fish under study, as the results of the current study showed that the Ramadi site showed a significant effect at a significant level ($P \leq 0.05$) Outperforming the Fallujah and Al-Baghdadi sites, the sites examined took the descending order of the value of Malone Day aldehyde (AL-Ramadi <Al-Baghdadi <Fallujah). As for the members, the liver recorded the highest value of Malone di-aldehyde at Al-Ramadi site with a value (0.2418 mg MDA / gm liver). The members took the descending order in relation to the value of Malone di-aldehyde (liver <gills> muscles).

***Keywords*:** Pollution Heavy metal, Oxidation (MDA), *Cyprinus carpio*

Introduction

Pollution of river and sea water with industrial wastes and agricultural activities all over the world is the subject of the hour and one of the most serious problems due to the great industrial progress. This problem first appeared in Japan because the Japanese relied on fish as a staple food in their meals, as it appeared in Europe for the same reason, where heavy metal elements are considered One of those pollutants (Gulfarz *et al*., 2001), Environmental pollution at the global level has led to the pollution of the fish environment to some extent by harmful substances, which are often the result of human activity and represented in agricultural activities such as fertilizers, pesticides, industrial effluents, solid and wastewater in water bodies, and the extent of pollution of fish with heavy metals in a place gives Conclusive indication of the pollution of the aquatic environment (Farkas *et al*., 2002). Heavy elements cause the formation of Reaction oxygen species and Free radicals that have harmful effects on living things (Tripthi and Gaur, 2004), Heavy metals cause the transformation of fats and proteins into the cell membranes, which impedes transport operations through the cell membrane, resulting in the cell not performing its functions as required (Knowles and Donaldson, 1990; Lawton and Donaldson,1991).

The accumulation of heavy metals causes damage to the tissues and physiological functions of the body of the organism as it stimulates the production of reactive oxygen such as hydrogen peroxide (H_2O_2) and lipid peroxide LOP (Firat *et al*., 2009). Fish, like other organisms, are susceptible to the effects of reactive oxygen (ROS) resulting from the oxidation process during metabolism under normal physiological conditions which can be eliminated by an antioxidant defense system such as an enzyme Catalase and Super dismutase (Romeo *et al*., 2000; Asagba *et al*., 2008).

If reactive oxygen production affects antioxidants, it can result in oxidative stress, leading to oxidative damage in several cellular targets **(**Lopes *et al*., 2001), As oxidative stress occurs as a result of the lipid peroxide and the high level of malone di-aldehyde (MDA) In the liver, which causes rupture of the membranes of the liver, which leads to leakage of fluids and enzymes outside the cells, and hence the high level of those enzymes in the plasma or blood serum (Dalkilic *et al*., 2015). In recent years, oxidative stress can be indicated by the level of lipid peroxides (LOP) And some criteria for the antioxidant defenses where the fat peroxides mechanism leads to the production of Malone Die Aldehyde (MDA) Which is the main oxidation product of unsaturated fatty acids where the abnormal increase is detected by measuring the levels of malone di-aldehyde (Remeo *et al*., 2000; Liu *et al*., 2008), In general, many of the biomarkers indicating oxidation, including enzymatic and molecular, are used to assess environmental risks (Van *et al*., 2003).

Area study

The carp were studied ***Cyprinus carpio*** Farms in cages on the Euphrates River within Anbar province, and three study sites were chosen, each site was considered a treatment and each fish is repeated. Before starting the research, it was confirmed that the breeders that the fish are from one breed and one age and from one source and the nutrition all cages include the same diet, which means it was under the same conditions, all samples were drawn in the summer, and the locations are as follows:

- Al-Baghdadi district, located more than 100 km west of the city center of Ramadi, in points GPS (42,5372660, 33,8578940) where a site for fish farming was identified in that region. The cages were randomly distributed irregularly and the weights at the beginning of the breeding period ranged between (200–250)gm The samples were drawn in the summer morning at ten o'clock and the air temperature was 39° C. and the water temperature 33° C. The average weights of the samples ranged between (1250–900)gm Where 6 bis were withdrawn from the beginning, middle and end of the cages and the cages were dimensions (4 x 4 x 2) m The

fish were transported in an ice box for the purpose of dissecting the fish and taking the tissues of the examination (liver, gills, muscles) to check the oxidation (malone di-aldehyde) and examine the heavy metals Water samples were drawn from a depth of (30 - 50) cm by means of special polyethylene bottles and kept in a container containing snow, for the purpose of carrying out chemical tests in the laboratories of the Great AL-Ramadi Water Project.

- The site of the cotton region of the city of Ramadi, which is about 10 km northwest of the city of Ramadi in points GPS (43,1561640, 33,5138650) This site contains 30 cages regularly installed two lines facing two dimensions (5 x 3 x 2) m For each cage and with a depth of 3 m for the cage, the weights at the beginning of the breeding period ranged between (300 - 400) g With a production volume of (2000-2500) fish / cage, the samples were drawn at ten in the morning, the air temperature was 41 °C and the water temperature was 32 °C, the sample weights were (850 - 1,500) gm , and 6 bis were removed from the fish and water in the same way above.
- The Nasaf site of the city of Fallujah, located east of the city of Ramadi, about 40 km in points GPS (43,6351600, 33,3719200) and contains 40 cages with dimensions (6 x 3 x 2) m For each cage distributed by two lines facing depth (2 m) and samples were taken in the morning and the air temperature and water temperature (33 °C) And the average weight of the beginning of the breeding period ranges between (200-300) g / fish and with a production size of (2000 - 2400) fish/cage.

Materials and Methods

Water physic – chemical measurements :

The temperature, pH and dissolved oxygen were measured locally by special portable devices to measure temperature, pH, and dissolved oxygen as shown in the image (2) The rest of the chemical properties are turbidity and total solid and conductive materials E.C. The total alkalinity and nitrate of TDS were measured in a laboratory in the Ramadi Grand Water Project laboratory, he indicated (Baird *et al*., 2012).

Estimation of the value of thiobutyric acid

Have used (Witte *et al*., 1970) To estimate the fat oxidation in tissues, by measuring the amount Malondialdehyde, Which is one of the products of the process of fat oxidation in different tissues, by estimating the value of theobiobutyric acid (TBA) Which are summarized as follows:

Puree 10 g of fish tissue sample (liver, gills, muscles) with 25 ml of solution containing trichloroacetic acid (20% concentration) (Trichloroacetic acid, TCA) And dissolved in phosphoric acid of standard (2) molar and for a period of (2 minutes), then transfer the mixture to a volume (50) ml volumetric flask, complete the volume of the mark with the distilled water and shake the mixture, It was taken (25) ml and was centrifuged at a speed (3000 rpm) for 30 minutes The mixture was filtered through a filter paper Whatman (No. 1) Then transfer (5) ml of the filtrate to a test tube and add (5) ml of reagent solution (TBA) (0.005M) dissolved in distilled water and prepare the control solution (Blank) Mix (5) ml of distilled water with (5) ml of TBA reagent solution. The contents of the test tubes were mixed well and closed tightly, and kept in a dark place for (15-16) hours at room temperature. The examination was carried out in the laboratories of the food Industry Department of the College of Agriculture – Anbar University and as shown in the picture (1, 2).

The absorbance (A) of the resulting color along a wavelength (530 nm) was measured using a spectrophotometer TBA By multiplying the absorbance value by the coefficient (5.2) and expressing the value TBA On the basis of the Malondialdehyde mmg / kg meat according to the following formula:

TBA value (MDA mg / kg meat) = A x 5,2 (dilution coefficient).

Statistical Analysis

Statistical analysis conducted one-way The trend included the influence of experiment sites on the studied traits, following the general linear model Using a program SAS Statistical Ready Edition 9,1 (SAS, 2002), And significant differences between the averages were tested using a test Duncan polynomial (Duncan, 1955) At the level of significance ($P \leq 0.05$) according to the following mathematical model equation:

$$Y_{ij} = \mu + T_i + E_{ij}$$

whereas :

Y_{ij}: j value of the observed attribute of the coefficient i.

μ: the overall average of the attribute.

T_i: effect of treatment i.

E_{ij}: a random error that is assumed to be a normal distribution with an average of zero and a variance of $\sum^2$.

Results and Discussion

The physical and chemical properties of the studied sites water :

Table (1) shows the physical properties of the water studied sites on the Euphrates River within Anbar Province, which includes Al-Baghdadi site to the west, the second site Al-Qutniya area of the city of Ramadi and the third site Al-Nasaf area of the city of Fallujah east of the city of Al-Anbar Governorate:

Table 1 : Shows the physical properties of the Euphrates River for the studied sites

Seq.	Parameter	Al-Baghdadi	AL-Ramadi	AL- Fallujah	Standard limits
1	Temp. of water	33	32	33	<35
2	PH	8.2	7.8	8.7	6.7- 8.5
3	D.O.(mg/L)	7.7	7.5	7.9	>5

In Table (2) the chemical and physical properties of the water studied on the Euphrates River within Anbar Province, which includes Al-Baghdadi site to the west, the second site Al-Qutniya area of the city of Ramadi, and the third site Al-Nasaf region of the city of Fallujah east of the city of Al-Anbar Governorate:

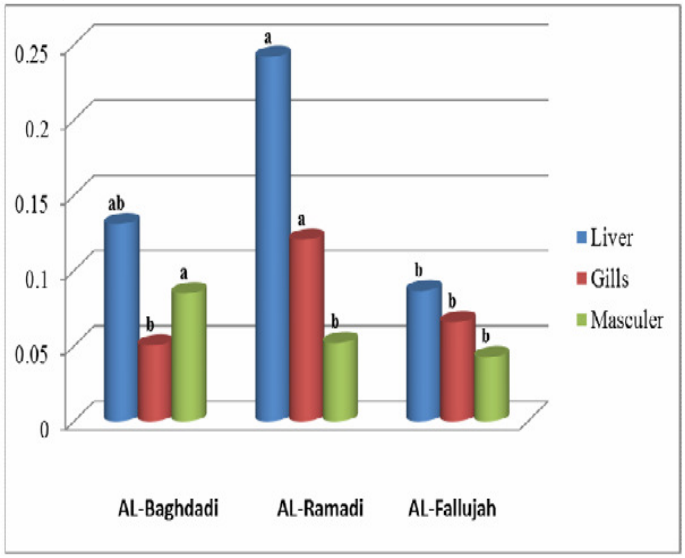
Table 2 : Shows the chemical properties of the Euphrates water for the studied sites

Seq.	Parameter	Al-Baghdadi	AL-Ramadi	AL- Fallujah	Standard limits
1	Turb.(NTU)	1.2	3.4	2.6	5
2	E.C.	680	646	740	-----
3	T.D.S	455	462	515	1000
4	T.H.	273	262	346	500
5	Alk.	168	165	87	250
6	NO3	18	15	21	40
7	CL	80	76	70	350
8	Turb.(NTU)	1.2	3.4	2.6	5

The measurements of this study came within the permissible limits according to the determinants of the river maintenance system from pollution and the standards of the Iraqi river water 1967 No. 25, and the standards of the World Health Organization (WHO, 2004). Although most of the sewers flow into the river course, the high amount of water and its severe runoff have a clear effect in reducing pollution, as the study showed that the physical and chemical specifications of the Euphrates water fall within the internationally and locally permissible limits and the results of this study are consistent with what came Each of (Al-Janabi, 2007), (Tahseen *et al*., 2009), (Rahul *et al*., 2012) and (Patil *et al*., 2013).

Malone Di aldehyde Test (MDA)

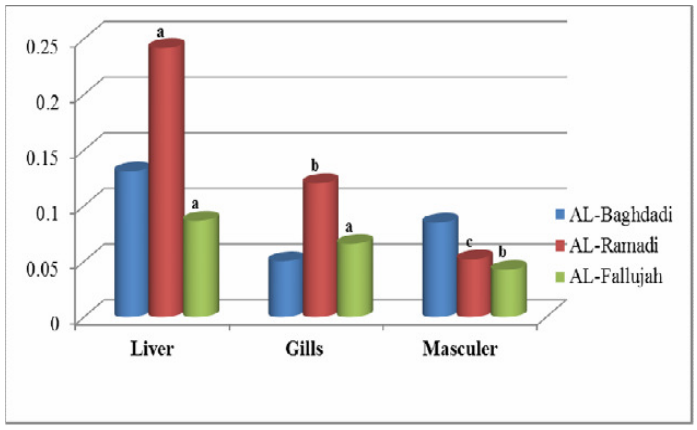
Figure (1). Influence of studied sites on the level of di-aldehyde malone in fish ***Cyprinus carpio.***



a,b, c: Different lowercase letters indicate significant differences between the studied sites at the level of significance (P≤0.05).

Figure 1 shows the results of the MDA test at the sites under study where the results of the statistical analysis showed that the Ramadi site showed a significant effect at the level of significance (P≤0.05) on the Al-Baghdadi and Fallujah sites on the values of the Malone di-aldehyde where the liver member scored in AL-Ramadi fishes are the highest value for (MDA) While the site of Fallujah was less affected by oxidation of fats at the level of significance (P≤0.05) as it recorded the lowest value of (MDA) For the studied sites, the sites took the descending order as follows : (Al-Ramadi <Al-Baghdadi <Al-Fallujah). In general, the values of Malone di-dehyde for all the studied sites were within the permissible limits, which is less than (2 mg Malone di-Aldehyde / kg) from the material under test.

Figure (2) effect of studied members on the level of Malone di aldehyde in fish *Cyprinus carpio* :

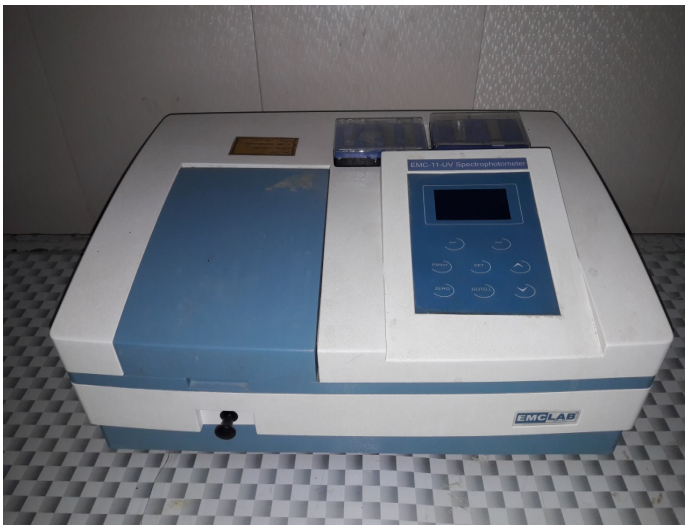


a, b, c : Different lowercase letters indicate significant differences between the sites studied at the level of significance (P≤0.05). Lack of letters means that there is no significant difference between the different members in the rate attribute because the values are dispersed from their mean, which leads to an increase in the value of the standard error.

Figure (2) shows the effect of the organs (liver, gills, muscles) of carp fish collected from the studied sites, the first site represents the Al-Baghdadi region and the second site in Ramadi and the third site represents the Fallujah region on the Euphrates River within Anbar Governorate, where the results of the statistical analysis showed the superiority of tissue The liver is in a gray site at a significant level (P≤0.05) , The highest value of (MDA) was recorded (0.2418 Malone dialdehyde/liver gm), whereas the tissue of gills exceeded the value of (MDA) at the Fallujah site at a significant level (P≤0.05), where the value of (MDA) of the drains was recorded (0.0658 Malone di aldehyde/Gills gm), As for muscle tissues, the muscles of the Fallujah site were more affected by the oxidation process, as the value of MDA (0.0424 Malone di-aldehyde/gm muscles) which is a natural reflection of the breakdown of peroxides, where the (MDA) is one of the by-products as the last and most stable result of the oxidation process By the action of the free radicals of unsaturated fatty acids It is also the best guide to measure the oxidation process (pokorny *et al*., 2001).

The importance of free radical reactions and reactive oxygen (ROS) reactions in the physiological processes of organisms in response to environmental pollutants stimulates the increased oxidative stress induced About these pollutants, especially heavy metals with oxidative damage in aquatic organisms, as evidence of environmental pollution with toxic substances (CELSO *et al*., 2003), Previous studies indicate that cadmium affects the antioxidant defenses in fish and it has been shown that it can compete with essential minerals at

binding sites with proteins where it stimulates increased release of iron ions Fe^{+2} and copper Cu^{+2} Causing increased reactive oxygen generation (Pruski *et al*., 2002), The members ranked descending in the level of Malone Die Aldehyde as follows: (Liver <gills <Muscles), and in a study he conducted (Risso *et al*., 2004) Lead did not report problems at average concentrations (0.05 mg / kg dry weight) of lead in the muscles of Mediterranean fish and predatory fish muscle, and did not find (Barak and Mason, 2004) Muscle effects of Roach fish were affected by the value of (MDA) collected from different locations in Brett, England that contained minerals (Cd, pb, Hg), The results of the current study were approximated with results (Jastrzebska, 2010) With regard to the elements of lead and cadmium in his study the effect of lead and cadmium contamination on *Cyprinus carpio* fish.



Picture 1 : Shown type of device which done Test (MDA) of this study.

Picture 2 : Shown some of carp fish tissues which done Test in this study.

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