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Toxicological Effects Of Heavy Metals On Histological Alterations In Various Organs Of The Red Mullet, Mullus Barbatus (Actinopterygii: Perciformes: Mullidae) From The Syrian Coast

Adib Saad And Yana Soliman
Scintific Research And Publishing Directorate, Manara University, Latakia, Syria
General Establishment Of Fisheries, Jable, Syria

Abstract:
This study was carried out to determine the concentration of some heavy metals (Cu and Pb) in the muscles and liver of the red mullet Mullus barbatus obtained from three sites of Syrian coast during 2021-2023 was investigated (atomic absorption spectrophotometry). And to study the histological changes of both the liver and the muscles as a result of the accumulation of heavy metals within them to clarify the effect of environmental stress on the Mullus barbatus. It was found that the metals were accumulated in different tissues of Mullus barbatus by various levels, where, the non-edible parts accumulated more metals than the edible muscles
The concentrations of copper and lead in the muscles were within the safe limits allowed by the World Food and Agriculture Organization, and therefore they do not pose a threat to the health of the human end-consumer. Several histological alterations were observed in the muscles, including elogation of muscle fibers. The liver showed vacuolar dilation in blood vessels, Hemorrhage, fatty degeneration, dilation of blood vessels and focal areas of necrosis. It was concluded that the environmental contamination of Syrian coast induced several histological alterations in the tissues of Mullus barbatus
Keywords: *histological alteration Mullus barbatus, heavy metals, liver, muscles Syrian coast.*

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I. Introudution:

Pollution of aquatic environment is a serious and growing problem (Alinnor and Obiji, 2010), due to increasing natural processes and anthropogenic activities. (Abalaka *et al*, 2020). Heavy_metals have become a serious issue now-a-days because of their bioaccumulation and toxicity tendency in the food chain leading to diseases in humans. (Fatima and Osmani, 2013). Fish is considered as a one of the vital sources of animal protein, especially low-income for millions of people worldwide. (Dohaish, 2018). Fish are generally considered to be an important bioindicator of aquatic environments, which usually constitute the last ring of the food chain, are considered to be one of the important groups for transferring metals to humans(Aytekin *et al*,. 2019). Harmful effects of pollutants can be manifested in fish tissues before consequential changes in fish behavior or external appearances (Mahboob *et al*., 2020; Nikalje *et al*., 2012).

Histological changes can be used as indicators for the effects of various anthropogenic pollutants on organisms and can be used as biomarkers to evaluate the overall health of the entire aquatic ecosystem.) Coulibaly *et al*., 2012; Saad *et al*., 2011). The effect of different contaminants on liver and muscles causing histological change have been analyzed in several studies (Mohamed, 2008; Kaoud and Dahshan, 2010; Alaa, 2012; Fatima *et al*. 2015 and Mohammed *et al.,* 2017., Sula *et al*., 2020). The presence of some heavy metals has been reported in Syrian coast (Ibrahim, 1999; Mohamad, 2001; Hammoud, 2005; Mohamad, 2007; Saad and Hammoud, 2007; Sarem *et al*., 2015; Hammoud and salama, 2016). However, there is currently no report on the assessment of heavy metals concentration in Syrian coast and their histological implications *Mullus barbatus*. , it is very economically important for Syria (Ulman *et al*., 2015), and it constitute a large part of the total seafood production in Syria (*Soliman et al*., 2016). The objectives of this research were: a) to determine the level of metals in the liver and muscles of : *Mullus barbatus* collected from Syrian coast b) to evaluate the histological changes in the liver and muscles of : *Mullus barbatus*

II. Materials And Methods

Study area:

The fish samples of were collected from the different sites a long Syrain Coast: The first site latitude (34° 59' " 46N 35°53' "21 E). It is located relatively far from the direct industrial pollution sources (T1). The second site latitude (35°10' "11N 35°55' "36 E). Was performed based on related to the thermicpower station activities (T2). The third site represents an area for the sewage downstream (T3) latitude (34 ° 53' " 09N 35°52' " 57 E) (Fig.1).

Fish Sampling and estimation of havey matals in the tissus:

Samples were collected from the Syrian coast (Fig. 1) during the seasons of 2021 to 2023. in order to determine metal in their tissues. . (*Mullus barbatus* n =48 individuals were collected and were analyzed from three station in order to determine metal in their tissues. The fishes were immediately placed on ice in an insulated box and transported immediately to the laboratory and stored at 20 C until analyzed. Total weight and Lengths for all individual was measured with the nearest 0.1 cm and 0.1 g. Tow organs(liver and sufficient amount of muscle) were dissected. The wet digestion method was used in the analysis of the heavy metals. Samples were transferred into digestion flasks and treated with 5 ml HNO3 (ultrapure, Merck) on the hot plate until the color turns into light yellow, nearly white. After this process the samples were transferred to 25 ml flanks and added double distilled water until 25 ml. The solution was filtered by filter papers. After digestion, all the samples were analyzed for the trace metal (Pb and Cu) concentrations using computer controlled Atomic Absorption Spectrophotometer (Spectra AA 220) Accuracy of the employed method was tested with a reference material

Histology:

We evaluated the liver and muscles of the sampled fish for Histological changes after harvest by fixing these organs in 10% neutral buffered formalin. These tissues were fixed in Bouin's solution for 24 hrs and then washed in running tap water and dehydrated through a graded alcohol series and embedded in paraffin wax. 2 section of tissues were cut using a rotary microtome and stained with hematoxylin and eosin. These sections were mounted with Canada balsam and cover slip was mounted on slides and placed in incubator for one night. Slides were examined under microscope and photographed at 10X-40X. These slides were studied for histological alterations in each organ and compared to the normal histological slides.

Statistical Analysis

Each reading represents Mean± SD of fish. Metal concentrations in different sites were analyzed by one-way analysis of variance (ANOVA). The level of $P \leq 0.05$ was regarded statistically significant.

III. Resultus And Discussion

The concentration of Heavy Metals: The accumulation of Copper and Lead in liver and muscle of, *Mullus barbatus* obtained from the three studied stations were shown in Table (1).

Concentration of heavy metals in fish tissues:

Copper (Cu)

In the liver, *Mullus barbatus* accumulated the highest concentration of Cu (9.91 ± 2.78 µg/g wet wt) (T3); while(T1) showed the lowest values (7.85 ± 3.11 µg/g wet wt). which were significantly higher than that of all stations. Concentrations of Cu in muscles ranged from 0.462± 0.10 (T1) to 0.691 ± 0.10µg/g wet wt (T2). Copper concentrations in muscles tissues found in this study were higher than other fish, Syrian coast (Hammoud and salama, 2016). And lower than *Chelon labrosus* from the southern part of Syrian Coast (Sarem *et al*., 2015**)**

Lead (Pb)

Concentrations of Pb in liver ranged from 0.02 ± 0.022 (T1) to 0.33 ± 0.035 µg/g wet wt (T3). while the concentrations of Pb in muscles ranged from 0.0281 ± 0.009 (T1) to 0.0.0778 ± 0.009 µg/g wet wt (T2). which were significantly higher than that of all stations

Lead concentrations in muscles tissues found in this study were lower than other fish, Syrian coast. Hammoud and Salama (2016). Lead concentrations in muscles tissues found in this study were lower than those Alexandria, Egypt. (khaled, 2004)

The maximum level of heavy metals was recorded at site 2 and 3 in all the organs, which received sewage wastes and Industrial wastes ,while lower values were recorded at site 1 which far from the direct pollution sources.

The highest levels of heavy metals were found in the liver, while lowest levels were found in the muscle that the muscle is not an active site for metal biotransformation and accumulation as reported by (Mohamed, 2008). Moreover, liver the first chance to metabolize these substances making it to be the first organ to be exposed to toxic compounds (Opute *et al.,* 2016).

The similar findings were reported by Hammoud (2005) and Sarem *et al.,* 2015 who found that higher values of the metal levels were observed in live and lower in muscle in other fish.

Histological alteration of muscle:

The muscle tissue constitutes the largest part of the fish body.(Yacoub *et al*., 2021) Its overall functions include locomotion, pumping of blood, synchronized movement of skeletal components, peristaltic constriction of visceral organs and their related structures (Kadry *et al*., 2015).

Histological study of muscle tissue of the control sites (T1) showed normal histological structures of the muscle and muscle bundles with equally spaced muscle bundles which indicated the fish to be in unstressed conditions. Figures 1(a)

Whereas the fish collected from polluted sites (T2-T3) showed fiber elongation Figures 2 (b) , fibrous bundles naturally Figures 2 (c)

These occurby severe degree in fish obtained from sites 2 that received industrial wastes. It is possible the that the changes in the muscles could be a direct result of the heavy metals, pesticides, fertilizers, salts and sewage (Mohammad, 2009).

The histological alterations in the muscles of Mullus barbatus are in agreement with those observed by many investigators who have studied the effects of different pollutants on fish muscles (Mohamed, 2008; Mohamed, 2009; Shahid *et al*., 2020 ; Yacoub *et al*., 2021).

Histological alteration of liver:

Histological study of liver tissue of the control site(T1) showed the normal histological appearance of the liver with hepatic cells, nucleus, sinusoids and blood vessels. Hepatocytes are polygonal in shape, arranged in several cellular layers and surrounded by sinusoids (S). Figures 3(a),In our study, several histological alterations were observed in *Mullus barbatus* liver from polluted sites (T2-T3). The histological changes found in the liver of fish collected from site (T2) included Hemorrhage around central vein and fatty degeneration ( )Fd) Figure 3(b) focal areas of necrosis in the liver of fish collected from site(T2) Figure 3(c), and dilation of blood vessels (db) and and focal areas of necrosis (nec) (T3)(Figure 3(d). That is explained by the fact why the liver is a major organ which performs metabolic functions, with one of the main functions being to clean pollutants from the blood coming from the intestine (El-Naggar *et al*., 2009), so it is considered as indicator of aquatic environmental pollution (Alaa, 2012). .Necrosis of hepatocytes may be due to the the cumulative effect of metals and the increase in their concentrations in the liver.

Hemorrhage in blood vessels may be due to fertilizers, salts and sewage discharged (Yacoub *et al*., 2021). Necrosis and hemorrhage may be due to the Toxins secreted by micro organisms in sewage water (Saad *et al.,* 2011).

Haemolysis observed in the blood vessels with subsequent responsible for the necrosis in the liver (Mohamed, 2009).

The present results are in agreement with those observed in other fish species under the influence of different pollutants (Fatima and Usmani, 2013; Kaur *et al*.,2018 ; Shahid *et al.,* 2020 ;Yacoub *et al*., 2021).

IV. Conclusion

It was found that the metals were accumulated in different tissues of *Mullus barbatus* by various levels, where, the non-edible parts accumulated more metals than the edible muscles. Cu and Pb concentrations in the fish muscles were low the maximum permissible limit. The results of this research indicated that the accumulation of heavy metal contamination caused many histological changes in the tissues of *Mullus barbatus*: It is useful to carry out studies in the future on the effect of heavy metals on the action of enzymes in the same current fish species and other fish species in the Syrian marine waters.

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Table 1. Average concentrations of heavy metals (µg/g wet wt) in various tissues of *M. barbatus* collected from the Syrian coast.

Tissue	site	Cu	Pb
		Mean Sd	Mean Sd
Muscle	T1	0.462 ± 0.10 a	0.0281± 0.009 a
	T2	0.619 ± 0.10 b	0.0651 ± 0.010 b
	T3	0.578 ± 0.13 ab	0.0778 ± 0.009 c
		Cv=11% Lsd=0.061	Cv=10% Lsd=0.002
Liver	T1	7.85 ± 3.11 a	0.02 ± 0.022 a
	T2	8.91 ± 2.66 a	0.26 ± 0.033 b

	T3	9.91 ± 2.78 b	0.33 ± 0.035 b
		Cv=6% Lsd=4.042	Cv=12.9%Lsd=0.043

Different letters (a, b, c) within a column indicate significant differences among sites (ANOVA $p < 0.05$).

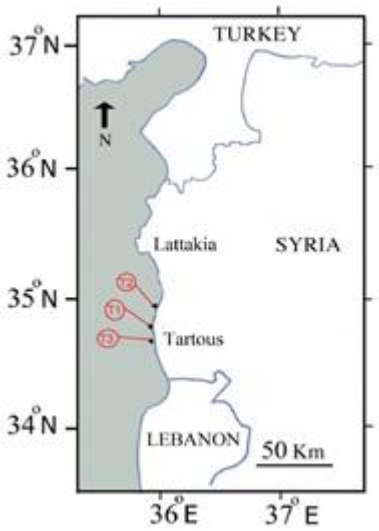

Figure 1. Map of the sampling areas in the Syrian coast.: T1, T2 and T 3 = Sites of fish sampling

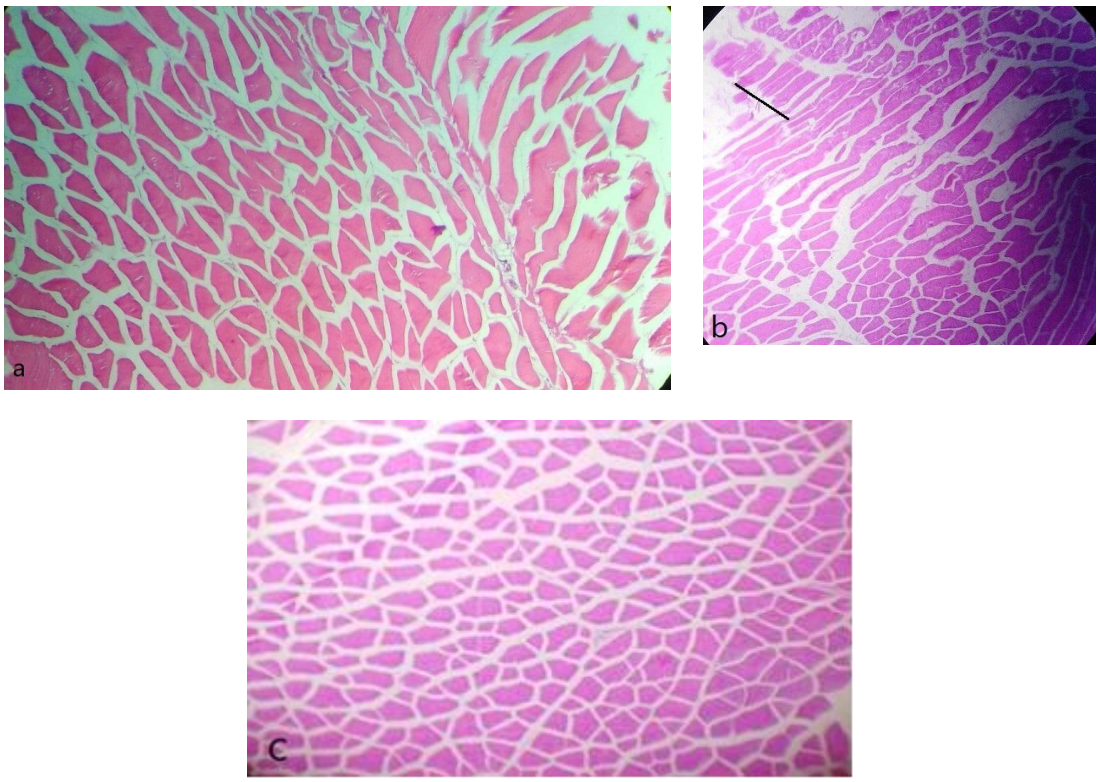

Figure 2. Muscles of fish showing the normal (a) (10X), fiber elongation (b) (10X), fibrous bundles naturally(c) (10X)

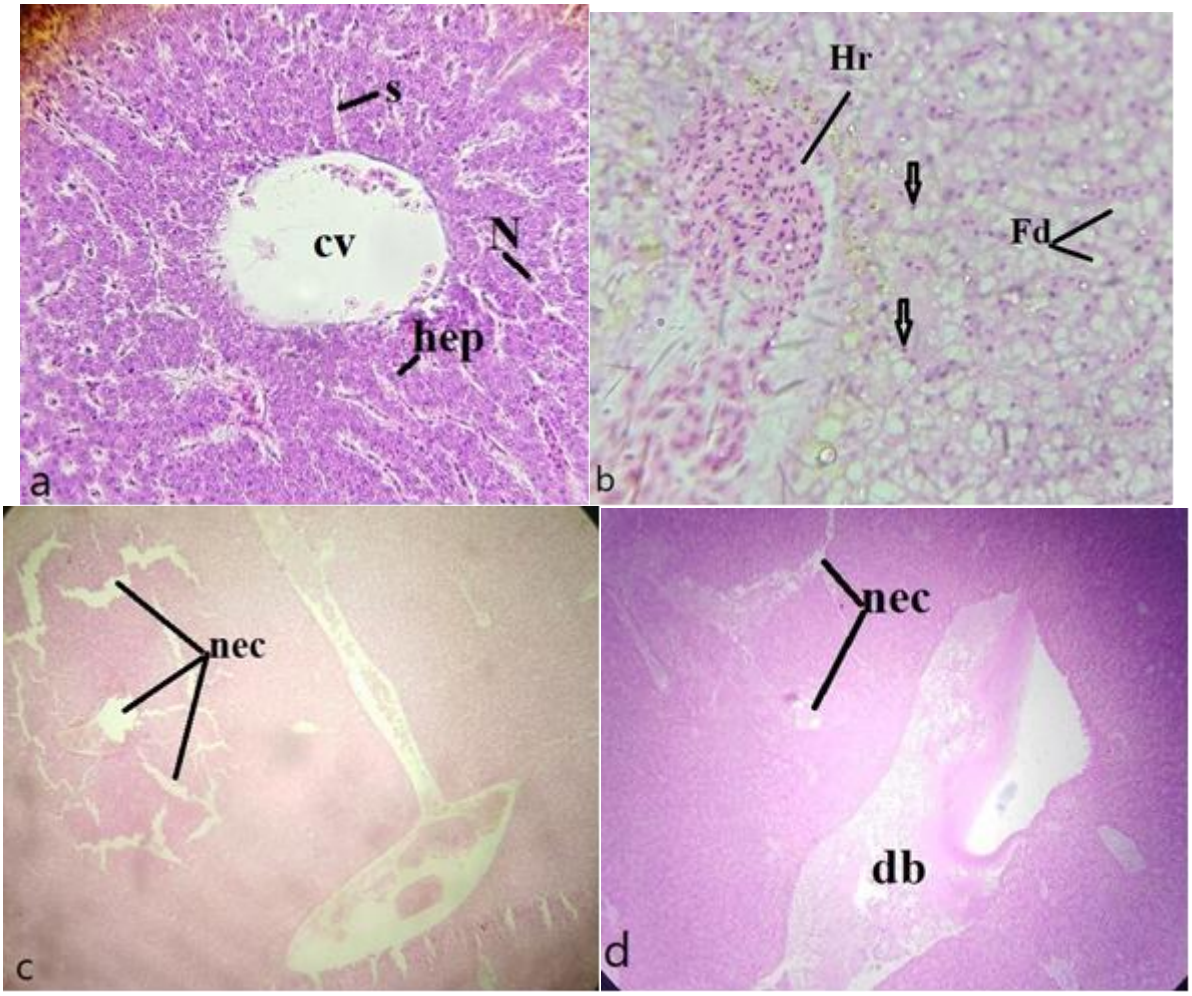

Figure 3. Liver of fish showing the normal the central vein (CV) is surrounded by cords of hepatocytes ,nucleus(N), sinusoids(S) (a) (40x), Hemorrhage around central vein and fatty degeneration) (Fd) (40x), (b), and focal areas of necrosis (nec) (c))X10). dilation of blood vessels (db) and and focal areas of necrosis (nec) (d)(10x),