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BIOACCUMULATION OF HEAVY METALS AND HISTOLOGICAL CHANGES IN TISSUES OF MARBLE SPINEFOOT (SIGANUS REVULATUS) FROM THE SYRIAN COAST (EASTERN MEDITERRANEAN)

YANA SOLIMAN [a], ADIB SAAD [a*], VIENNA HMMOUD [b] AND ALI BASSAL [b]

[a] Marine Sciemces Laboratory, Faculty of Agriculture, Tishreen University, Lattakia, Syria.
[b] Department of Zoology, Faculty of Sciences, Tartus University, Syria.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to determine the concentrations of tow heavy metals, copper (Cu) and lead (Pb), and histopathological lesions in tissues of Marble spinefoot *Siganus revulatus* **(**Forsskål & Niebuhr, 1775) caught from the Syrian coastal waters during 2019-2020. Results indicated tha the metals were accumulated in different tissues of *siganus rivulatus* by various levels, where, the non-edible parts accumulated more metals than the edible muscles. Cu and Pb concentrations in the fish muscles were lower the maximum permissible limit, however, Cu in the liver exceeded the permissible limit. Several histological alterations were observed in the muscles, including vacuolar degeneration in muscle bundles, balloon necrosis, Melano- macrophage centers, splitting of muscle fibers and atrophy of muscle bundles. The liver showed vacuolar dilation in blood vessels, leucocytes infiltration, thrombosis formation in hepatoportal blood vessels haemorrhage, haemosiderin and focal areas of necrosis. It was concluded that the environmental contamination of Syrian coast induced several histological alterations in the tissues of *siganus rivulatus*.

Keywords: Histopathology; *siganus rivulatus*; heavy metals; bioaccumulation ; syrian coast.

1. INTROUDUTION

"Pollution of aquatic environment is a serious and growing problem [1], due to increasing natural processes and anthropogenic activities" [2]. 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 [3]. "Fish is considered as a one of the vital sources of animal protein, especially low-income for millions of people worldwide" [4]. "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" [5]. "Harmful effects of pollutants can be manifested in fish tissues before consequential changes in fish behavior or external appearances" [6,7].

"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" [8,9]. The effect of different contaminants on liver

**Corresponding author: Email: adibsaad52@gmail.com;*

and muscles causing histological change have been analyzed in several studies [10-15]. The presence of some heavy metals has been reported in Syrian coast [16, 17, 18, 19, 20, 21, 22]. However, there is currently no report on the assessment of heavy metals concentration in Syrian coast and their histological implications in *siganus rivulatus.*, it is very economically important for Syria [23], and it constitute a large part of the total seafood production in Syria [20]. The objectives of this research were: a) to determine the level of metals in the liver and muscles of : *siganus rivulatus* collected from Syrian coast b) to evaluate the histological changes in the liver and muscles of : *siganus rivulatus*.

2. MATERIALS AND METHODS\

2.1 Study Area

The fish samples were collected from a different three sites on the Syrian 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 on the therm power station activities (T2). The third site represents an area for the sewage downstream (T3) latitude (34 ° 53' " 09N 35°52' " 57 E) (Fig.1).

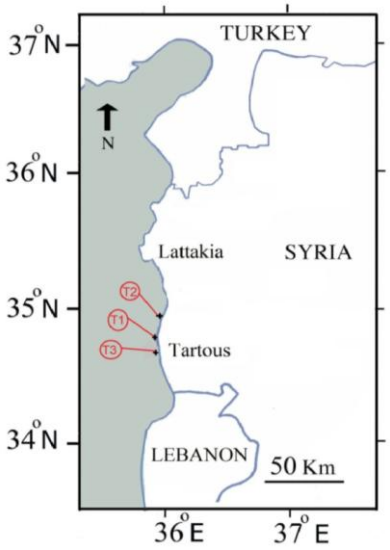

Figure 1. Map of the sampling areas in the Syrian coast.: T1, T2 and T 3 = Sites of fish sampling

Fish Sampling and estimation of havey metals in the tissus: Samples were collected from three sites on the Syrian coast (Fig. 1) during the seasons of 2019 to 2020. in order to determine the metal concentration in the fish tissues. Twanty four individuals were collected and analyzed. The fishes were immediately placed on ice in an insulated box and transported to the laboratory than 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: Tissue samples of musles and liver were quickly removed, fixed in 10% neutral buffered formalin during one monthe. Than, 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.

2.2 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.

3. RESULTUS AND DISCUSSION

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

3.1 Concentration of Heavy Metals in Fish Tissues

3.1.1 Copper (Cu)

In the liver, *S. rivulatus* accumulated the highest concentration of Cu (58.56 ± 5.85 µg/g wet wt) (T2) which received sewage wastes; while(T1) showed the lowest values (17.11 ± 6.45 µg/g wet wt). which were

significantly higher than that of all stations. Concentrations of Cu in muscles ranged from 0.392± 0.10 (T1) to 0.788 ± 0.130µg/g wet wt (T3) which received Industrial wastes. "Copper concentrations in muscles tissues found in this study were higher than other fish, Syrian coast [22]. And lower than *Chelon labrosus* from the southern part of Syrian Coast" [21]. El-Moselhy et al. [24] in Red sea Egypt, while the other herbivore (*S. rivulatus*) showed minor variations (as high concentrations of Cu in liver).

3.1.2 Lead (Pb)

Concentrations of Pb in liver ranged from 0.1 ± 0.022 (T1) to 0.231 ± 0.035 µg/g wet wt (T3). while the concentrations of Pb in muscles ranged from 0.01425 ± 0.001 (T1) to 0.022 ± 0.002 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 [22]. Lead concentrations in muscles tissues found in this study were lower than those Alexandria, Egypt [25].

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 [26]. Moreover, liver the first chance to metabolize these substances making it to be the first organ to be exposed to toxic compounds [27]. Cu in liver was found in high concentrations in samples of *S. rivulatus*, exceeding the limit (30) specified by FAO [28].

The similar findings were reported by Hammoud [22] and Sarem et al. [21] 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" [29].

Histological study of muscle tissue of the control sites (T1) showed a normal histological structures of the muscle and muscle bundles with equally spaced muscle bundles which indicated the fish to be in unstressed conditions. (Fig 2;a-b).

Whereas the fish collected from polluted sites (T2-T3) showed vacuolar degeneration in muscle bundles and balloon necrosis (Bn) (Fig 2;c). Also, Melano-macrophage centers (Fig 2;d). splitting of muscle fibers (Fig 2;e). And atrophy of muscle bundles were seen. (Fig 2;f).

"These occurby severe degree in fish obtained from sites 2 that received industrial wastes and sites 3 that received sewage 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" [26].

"The histological alterations in the muscles of *siganus rivulatus* are in agreement with those observed by many investigators who have studied the effects of different pollutants on fish muscles" [10, 26, 30, 29].

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

Tissue	site	Cu	Pb
		Mean Sd	Mean Sd
	T1	0.392 ± 0.10 a	0.01425± 0.001 a
Muscle	T2	0.730 ± 0.10 b	0.022 ± 0.002 c
	T3	0.788 ± 0.13 b	0.192 ± 0.002 b
		Cv=9% Lsd=0.061	Cv=9% Lsd=0.001
	T1	17.11 ± 6.45 a	0.1 ± 0.022 a
Liver	T2	58.56 ± 5.85 c	0.207 ± 0.033 b
	T3	45.77 ± 5.94 b	0.231 ± 0.035 b
		Cv=5.8% Lsd=3.042	Cv=12.9%Lsd=0.033

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

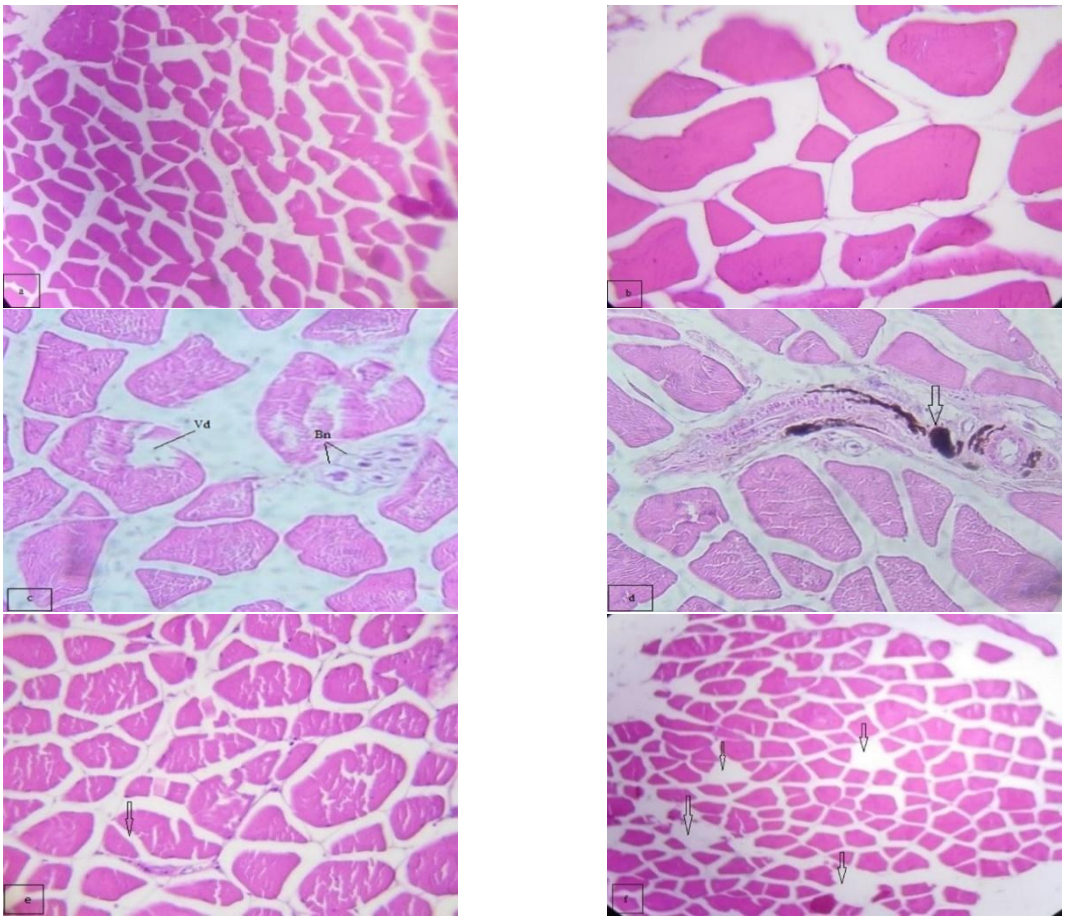

Fig. 2. Muscles of fish showing the normal (a-b) (X-10X40), vacuolar degeneration (VD) and balloon necrosis (Bn) (c({X40}), Melano - macrophage centers (arrows)(d)(40X), splitting of muscle fibers(arrows) (e ({40x), and atrophy of muscle bundles (arrows) (f)(10X)

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). Figs 3(a),In our study, several histological alterations were observed in *Siganus rivulatus* liver from polluted sites (T2-T3). The histological changes found in the liver of fish collected from site (T2) included dilation in blood vessels and leucocytes infiltration Fig. 3(b), thrombosis formation in hepatoportal blood vessels was observed and haemosiderin Fig. 3(c), haemosiderin was seen around central veins and hepatoportal blood vessels hemorrhage Fig. 3(d-e), and focal areas of necrosis in the liver of fish collected from site(T3(Fig. 3(f). "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 [31], so it is considered as indicator of aquatic environmental pollution" [12]. The alterations in histoarchitecture of liver were probably due to the accumulation of heavy metals i.e. copper (45.77- 58.56) ppm beyond the certified limits as shown in Table (1).Necrosis of hepatocytes may be due to the the cumulative effect of metals and the increase in their concentrations in the liver.

Hemorrhage and hemosiderin in blood vessels may be due to fertilizers, salts and sewage discharged [29]. Necrosis and hemorrhage may be due to the Toxins secreted by micro organisms in sewage water [9].

Haemolysis and thrombosis formation observed in the blood vessels with subsequent responsible for the necrosis in the liver [26].

The thrombus formation within blood vessels that might occur due to the platelet aggregation [32].

"The present results are in agreement with those observed in other fish species under the influence of different pollutants" [3, 33, 34, 29].

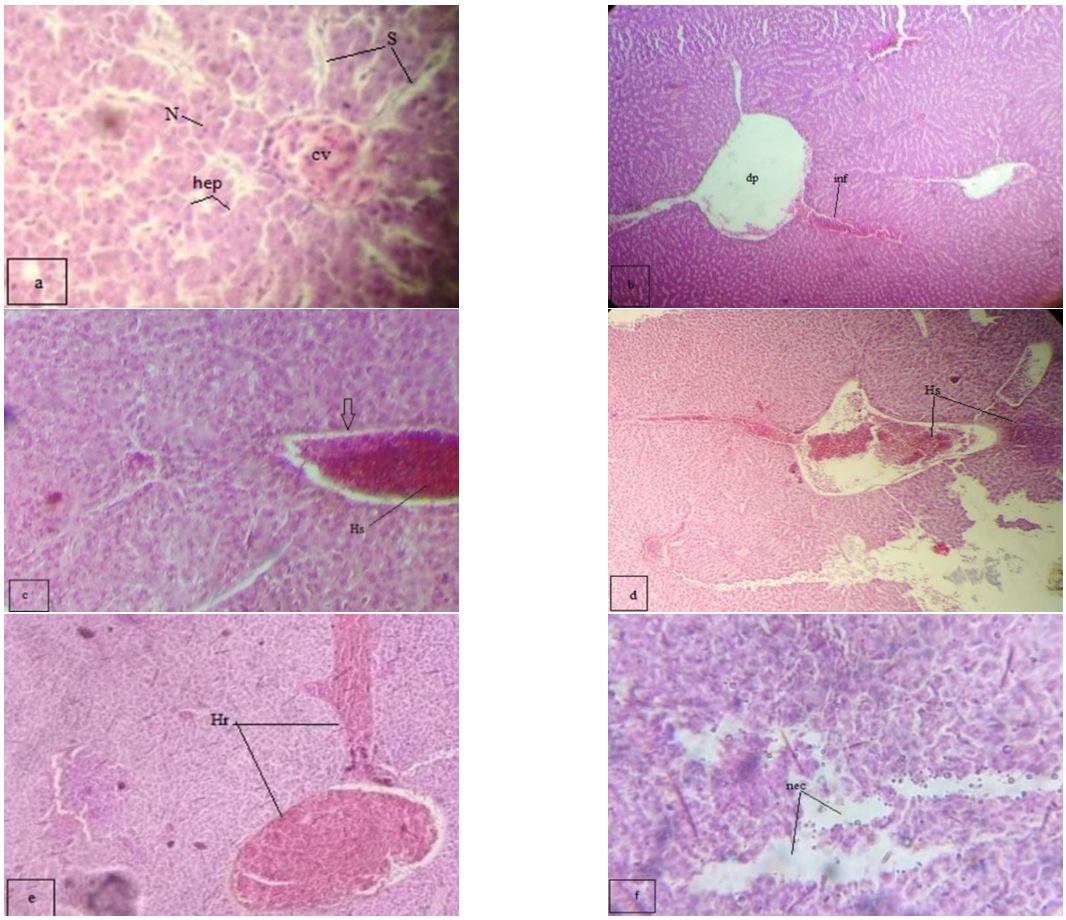

Fig. 3. Liver of fish showing the normal the central vein (CV) is surrounded by cords of hepatocytes, nucleus(N), sinusoids(S) (a) (40x), dilation of blood vessels (db) and leucocytes infiltration (inf) (b)(10x), thrombosis formation in hepatoportal blood vessel (arrows) and haemosiderin (10x), haemosiderin around central vein and hepatoportal blood vessel (d) (10x),intravascular haemolysis in blood vessels (e)(10x), and focal areas of necrosis (nec) (f))X40)

4. CONCLUSION

It was found that minerals accumulated in different tissues of siganus rivulatus at different levels, as the inedible parts accumulated more minerals than in the edible muscles. Cu and lead concentrations in fish muscle were below the maximum allowable, however, copper in liver exceeded the permissible limit. On the other hand, the results of this study indicated that the accumulation of heavy metal contamination caused many histological changes in the tissues of siganus rivulatus: it is useful to conduct future studies on the effect of heavy metals on enzyme action in the same current fish. Species and other fish species in Syrian marine waters.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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