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THE HISTOLOGICAL AND PHYSIOLOGICAL ALTERATIONS IN GILLS AND ERYTHROCYTES OF *CYPRINUS CARPIO* AND *LIZA ABU* AS BIOMARKER TO FRESHWATER POLLUTION

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ABSETRACT

As a component of a large research programme to evaluate the effects of contaminants on fish health in the fields , histopathological studies have been conducted to help establish meat of causal relationships between contaminants exposure and various biological responses and use of those responses as biomarker to aquatic pollution . Two fish species *C. carpio* and *L. abu* were sampled from four sites near of industrialize and human pollution areas from Shatt Al-Arab River ,while the fifth place was considered as control because it's far located from direct pollution sources . Some of histological and physiological alterations were examined in both gills and blood . The alterations were significantly higher in fish collected from two places (1 & 2) compare with other sites , histological was represented by hyperplasia , hypertrophy , bleeding , inflammations , edema , necrosis , epithelial separation and clubbing shaped , while , physiological was represented by decrease of Na^+ , K^+ , Ca^{+2} concentrations , Chloride cells number and increase of respiratory diffusion distance , in addition to , increase of micronuclear of red blood cells . all fish in fifth place didn't showed any histological and physiological responses and retained their rates of the normal level compares with other places .

INTRODUCTION

Biomarker research has been widely applied in field of environmental hazard assessment (1), also , biomarker have increasingly been used not only as effective tools for environmental monitoring , but also as early warning sentinels in ecological risk assessment (2) . There was little studies about effect of environmental pollutants on some of physiological and histological alteration in fish gills and erythrocytes and use of theses alteration as biomarker for freshwater pollution (3) . The freshwater often carries many pollutants which usually come from different sources such as the agriculture fields , factories , electricity power stations ..... etc. (4) .

Fish gills are important organ because it have manifold functions such as the exchange of gas and ions, acid and base balance and nitrogenous excretion (5) . Gills usually are provide most of extensive interface of the fish with the aquatic environment . The respiratory surface is covered only by a thin epithelium , and forms a barrier between fish's blood and surrounding water (6) , however , the gills contain different cells types such as chloride ,

parament and mucous cells (7) . Most of dissolved pollutants enter fish's blood via gills (8) , and so it will be the first organ which affected by aqueous exposure to toxicants (9) . In gills , many of histological and physiological alterations have been shown to be useful tools to characterize the health status of fish (10) , also , these alterations were used as an indicator for sublethal effects by different toxicants (11) , and assess of impacts of environmental contaminations on aquatic organisms which be in direct contact with the aquatic pollutants (12) , However, ionic and osmoregulation , number and function the chloride cells may be effected by exposure to aquatic pollutants (13) .

Histological alteration in gill showed different histopathological symptoms such as necrosis , fusion and edema ...etc. (14) .

The genetic mutagens contain wide range of chemical materials , which have ability to cause carcinogen diseases of fish . The many of cytogenetic studies in aquatic environment used the fish and showed that chromosomes aberrations and sister chromatid exchanges were induced by exposure to genotoxic agent in water, however , many of micronuclear in erythrocytes were resulted by chromosomes broking after exposure to different chemicals such as heavy metals , oil hydrocarbonate , pesticides and organochemicals (15) .

In the aquatic environment , fish was considered as to be a suitable organisms choice as biomarker for environmental pollution and located at the top of aquatic food chain as they have high ability to accumulate toxicants (16) .

In this study histological and physiological alterations in gills and number of micronuclear in RBC were investigated in two species of freshwater fish (*Cyprinus carpio* and *Liza abu*) . All these fish were collected from five stations located on Shatt Al-Araib River bank , the sites 1 , 2 , 3 and 4 are locate near directly discharge sources for different pollutants , while site 5 used as control because it's locate far with any directly pollution .

MATERIALS AND METHODS

Sample collection and handling

All fish sample (*C. carpio* and *L. abu*) were collected of Aprial 2005 from five sites in Basrah – Shatt Al-Arab River , four sites were located near some of pollution sources (Fig. 1) .

1- Site (1) is near the electric power station of Harthaa .
2- Site (2) is near the papers factory .
3- Site (3) is near electric power station of Najebia
4- Site (4) is near of discharge sources of Kandic canal.
5- Site (5) is in south Shaat Al-Ariab River which regarded as control treatment .

At all sites the temperature was approximately (28 ± 1.5) C^0 , pH (7.7 ± 0.35) and hardness (630 ± 45.5) .From each site , fifteen fish from each species were collected by cast

net . The fish were approximately (55 ± 5.3 and 38.45± 3.74) g weight for (*C. carpio* and *L. abu*) respectively .

Fish were transited to laboratory and kept overnight in water from breath environmental which they had been caught without any supply of food , afterwards , they were dissected and performance of histological and physiological tests .

Histological analysis

Directly after killing of fish , gills were isolated from the section of secondary left arch and fixed in (10 % con.) formalin solution . After 24hr., processing of dehydrating and clearing was preformed with alcohol and xylene , afterward , the tissues specimens were impregnated and embedded in paraffin wax . All the sections were preparated by the microtome (6 micrometer thickness)and stained with hematoxylin and eosin (17) .

Physiological analysis

The middle parts of secondary left arches of gills were isolated from live fish , and placed in puffer solution (sodium chloride 0.98 mg\l) during less than fifteen seconds , however , count of chloride cells were detected according to (18) . The Na^+ , K^+ and Ca^{+2} ions concentrations were measured in gills by Flim Photometer (ANA-10AL) after separating of ions from tissue by use of nitric acidic (19) .

The respiratory diffusion distance in gills was determined in all fish by use of microocular measurement (9) .

The blood samples were taken from caudal vasculature with a heparinzed syringe for count Micronuclear in erythrocytes . Smears of blood was prepared on slide and fixed overnight in methanol solution and stained by Gemssa stain (20) .

RESULTS

Histopathological changes :-

The morphological shape of gills filament is similar among the different fish species (21) . The histological structure of normal gills was consist of primary and secondary respiratory lamellae which covered by an epithelial layers, as it contains chloride , pillar and mucosa cells and blood sinuses (Fig. 2) .

Several histopathological symptoms in filaments epithelium of the primary and secondary lamellae were showed in fish which collected from (Site 1 , 2 , 3 and 4) . Fish's gills which captured at the site 2 and 4 was showed strongest damage in primary and secondary lamellae , while , slightly damage was observed in site 1 and 3 , however , hyperplasia , hypertrophy , necrosis , edema and acute degeneration , were observed in secondary lamellar epithelium (Fig. 3 , 4 , 5 and 6) , also , bleeding , congestion and inflammation states, as well as , fusion of neighboring secondary lamellae , hyper pigments ,

epithelial separation and clubbing shapes were determined (Fig. 7 , 8 , 9 , 10 and 11). In site 5 , fish's gills did not show any histopathological symptoms .

Physiological changes :-

Level the ions concentration (Na^+ , K^+ and Ca^{+2}) in gills was listed in table (1) . The ions concentrations were showed vacillant in different sites , the lowest were determined in site 4 for both fish species compared with other sites , while , the highest were found in site 5 which it's used as control location . A significant decrease ($P< 0.05$) in ions level was recorded in all specimens from site 2 and 4 compared with site 5 for both fish , while , the Ca^{+2} ions appeared significant decrease ($P< 0.05$) only in the sites 1 and 4 for *C. carpio* fish but in sites 2 and 4 for *L. abu* compared with site 5 .

The chloride cells number in gill filaments was represented in table (2) , Significant elevations ($P<0.05$) was recorded in the fore sites compared with site 5 for both fish . The values highest were found in site 2 and 4 compared with other sites .

Respiratory diffusion distance in secondary filaments appeared significant increasing ($P<0.05$) in the fore sites compared with site 5 in both fish . The highest values was showed in site 2 and 4 , while , the lowest values were recorded in site 5 compared with other sites .

In all fish the micronuclear number in sites 1 , 2 , 3 and 4 were significantly higher ($P<0.05$) comparedwith site 5 . the higher values were recorded in site 2 and 4 compared with other sites (table 2) .

DISCUSSION

In this study many of biomarker was represented the different of physiological and histological responses in gills , these alterations were observed in two fish (*C. carpio and L. abu*) which were from five sites of Shatt AL-Arab River .

Chemical analysis and limnlogical investigations in many studies confirmed that some of pollutants such as heavy metals , pesticides , hydrocarbonate and organic or inorganic materials were presented in four models (sites 1 , 2 , 3 and 4) , sites 3 and 4 were appeared highly pollution by different pollutants compare with sites 1 and 2 (22and 23) .

The present study aimed to assessing the suitability of histological and physiological responses as biomarker for water pollution .

Gills tissues was selected as biomarker for water pollution because they are representing useful tools for limitation the health state to aquatic system (24) . In fact the chemical analysis of water and sediment alone often was insufficiently guide in limitation of pollution level in aquatic environment , however , theses analysis often was represented only the results obtained at a given time point (25) .

Gills represent the first organ which affected by exposure to aqueous system toxicant because the directly content them to external environment (8) . Histological and physiological alterations in gills has been as reflection to different environmental conditions (

26) and Hypersensitive to aquatic pollutants (27) , however , many of local studies showed that the water and sediment in sites 3 and 4 were have higher pollution levels because increase in level the toxic compounds e.g. heavy metals , hydrocarbonate , pesticides and sewage discharge ....etc. , while the slighter pollution was showed in site 1 and 2 compared with site 5 (22 and 23) .

Gills of fish in sites 1 , 2 , 3 and 4 appeared with various in histological and physiological effects . These effects were classified to strongly and slightly alterations in sites 3 , 4 and 1 , 2 respectively , Generally , theses effects could be detected in epithelial of primary and secondary filaments of gill , respiratory diffusion distance , chloride cells and Na^{+} , K^{+} and Ca^{+2} ions .

Histological responses

The microscopic examinations was revealed each of hyperplasia in epithelial surfaces of respiratory lamellae , hypertrophy in cells of secondary branchioles which often was associated with increase in respiratory diffusion distance , proliferation in chloride cells , clubbing lamellae shape , vascular stasis or congestion , hyperpigment , separation and necrosis in epithelium and fusion of secondary lamellae . All theses pathological symptoms were more apparent in fish's gill of sites 2 and 4 compare with sites 1 and 2 , while , don't regard any changes in fish's gills of site 5 .

The hyperplasia and fusion of secondary lamellae may be caused by increase in inflammation of lamellae cells and exudative inflammatory(28) . Many researches were emphasized that the acute inflammation usually cause separation in lamellae epithelial and destruction in ionic and osmoregulation function (29) and (30) . Cells swelling in secondary lamellae may cause the water diffusion from external environmental into inside of secondary lamellae cells , this state also may be reasoned of disturbance in ionic and osmoregulation function after showed of edema state and destroy some of secondary lamellae cells (31) .

Hyperplasia and hypertrophy of secondary lamellae could be often observed in animals exposed to different chemical compounds in water and sediments , some of these symptoms often lead to the completely fusion in two or more of neighboring secondary lamellae (32 and 33) . Hyperplasia is considered the primary reason in increment of respiratory diffusion distance between the blood and oxygen rich water , finally this state may lead to an insufficient the oxygen supply to blood (29 and 30) .

Some of inflammatory reactions was detected in some of gill tissues from sites 3 and 4 , however , these inflammatory reactions may be arrived as result of exposure to stress because the direct contact for epithelial tissues with aquatic contaminations (34 and 26) .

Most of gills filaments in experiment fish which collected from sites 3 and 4 was showed that swelling as clubbing , occurred at the many of secondary lamellae tips with congestion in gill's blood sinuses . However , the exposure to different chemical compounds

and for long times may cause the progression of cells swelling towards of lamellae bases . The thrombosis in the many of secondary lamellae had been turned into club shape (35) .

Physiological responses

Chloride cells showed increment in the number in sites 1 , 2 , 3 and 4 compared with site 5 , the values were found higher in site 2 and 4 compared with other sites , however , this phenomenon was confirmed by several additional experiments (14 and 36) . The proliferation in chloride cells may be well documented responses to toxic exposure to different chemical compounds because the ability of cells to uptake of different ions by bronchial (36) . These agents including many toxic chemicals compounds (37 and 33) , however , functions of chloride cells is transport of different ions such as Na^+ , K^+ and Ca^{+2} between external environment and fish , therefore , an increment in chloride cells number may be interpreted as an adaptive response to compensate the ions losses because of stress resulted by exposure to aquatic contaminations (14) .

(38) and (39) suggested that increase of chloride cells number may resulted by disproportionate in proliferation of chloride cells by the increment in immature or degenerative cells , so that they will be not able on engagement (pursuit) the normal function and consequently , dysfunction in of ionic and osmotic homeostasis was recorded . (40) and (41) showed that the exposure to different concentrations of aquatic chemical compounds may cause increase of gills permeability to water and salts , this state will result decline in ions concentrations such as Na^+ , K^+ and Ca^{+2} and by the result will cause dilution states by water and movement facilitation of ions from intercellular fluids to extracellular fluids (42) . Many studies showed that most of organic and inorganic chemicals in aquatic system have inhibitory effects on the carrier ATPase (Na^+ , K^+ and Ca^{+2}) enzymes (43 , 44 and 41) .

Although all fish in sites 1 , 2 , 3 and 4 showed increase of micronuclear of RBC compared with site 5 however, the sites 2 and 4 appeared the higher effect compared with other sites . Micronuclear in erythrocytes may occur as a consequence of chromosome breakage and spindle dysfunction . Hydrocarbonate class and heavy metals are important groups in environmental mutagens \ carcinogens which has been implicated in the development of tumors among of fish populations (45 and 46)

Most the abnormalities nuclear in erythrocytes were showed in bullheads and mud minnows fish which were exposed to different from genotoxic chemicals (47) . In fact the determination of micronuclear was enumerated as a indicator on the genotoxic activity , (48) showed that greater incidence of mutagen was caused abnormality micronuclear in fish erythrocytes after exposure to different aquatic contaminations . In fact most of fish body cells have slower rates from DNA repair compares with other vertebrates , so that these cells will be more effected by the aquatic toxins and which caused clastogenic in erythrocytes ,

therefore , the long-term exposure to genotoxic agents may increase the frequencies of micronuclear in circulating erythrocytes in fish (47) .

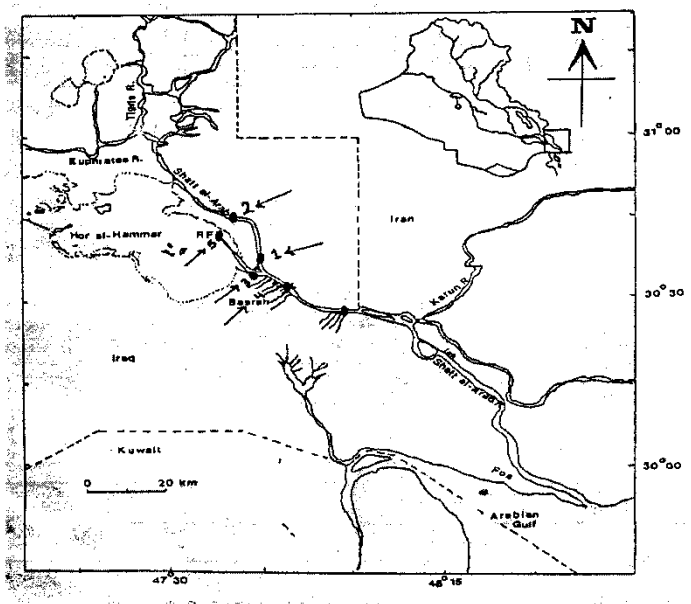

Figure (1) Sites of specimens collection (⟶)

Fig 2: Normal gills of the *C. carpio* (site 5) show normal filaments and Epithelium . ((→)) H.E. (400X)

Fig 3 : Gills of the *C. carpio* (site 3) show the hyperplasia of filaments (a) , degeneration of epithelium (b) , necrosis (c) and inflammation (d) , H.E. (400X) .

Fig 4 : Gills of *L. abu* (site 2) show a hyperplasia in filaments (a) , epithelium separation (b) , and fusion of neighboring secondary lamellae (c) , H.E. (400X) .

Fig 5 : Gills of *C. carpio* (site 4) show that edema (a) bleeding (b) , necrosis (c) , congestion of blood sinuses (d) and epithelial separation (e) , H.E. (400X) .

Fig 6: Gills of *L. abu* (site 2) show that inflammation (a) , hyperplasia (b) and necrosis of epithelium (c) , H.E. (400X) .

Fig 7 : Gills of *L. abu* (site 2) show that fusion of neighboring secondary lamellae (a) and congestion of blood sinuses (b) , H.E. (400X) .

Figure (8) Gills of *C. carpio* (site 1) show that fusion of secondary lamellae (a), epithelium necrosis (b) and congestion of blood sinuses (c), H.E. (400X).

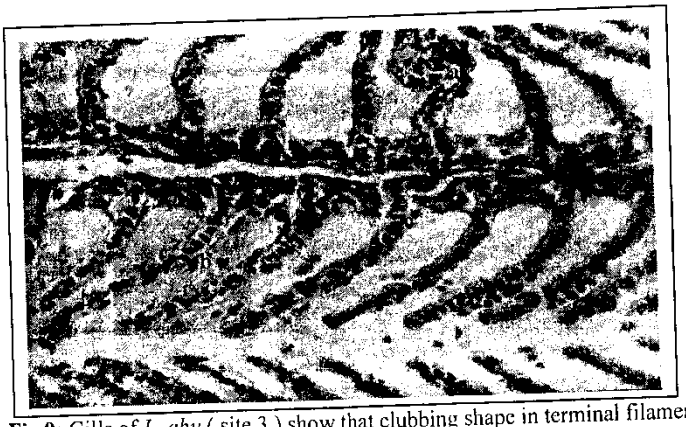

Fig 9: Gills of *L. abu* (site 3) show that clubbing shape in terminal filaments (a), epithelium separation (b) and congestion of blood sinuses (c), H.E. (400X).

Fig 10: Gills of *C. carpio* (site 1) show that epithelium necrosis (a) , congestion of blood sinuses (b) , separation of epithelium (c) , hyperplasia of epithelial (d) and cells hyper pigment (e) , H.E. (400X) .

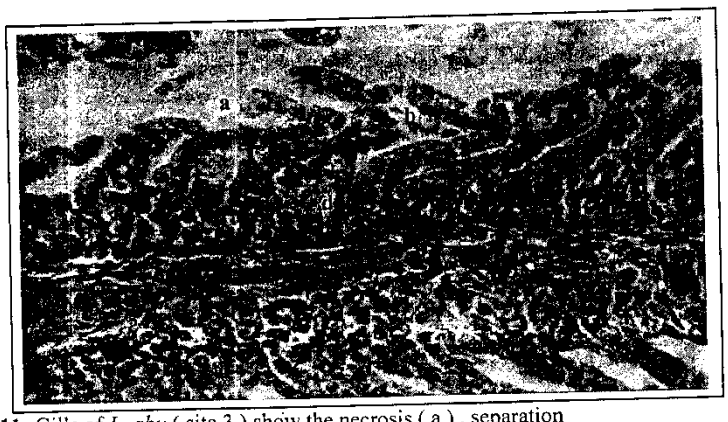

Fig 11: Gills of *L. abu* (site 3) show the necrosis (a) , separation (b) , hyperplasia of epithelium (c) and cells degeneration (d) , H.E. (400X) .

Table (1) Number of gills chloride cells , RBC micronuclear and respiratory diffuse distance some of fresh water

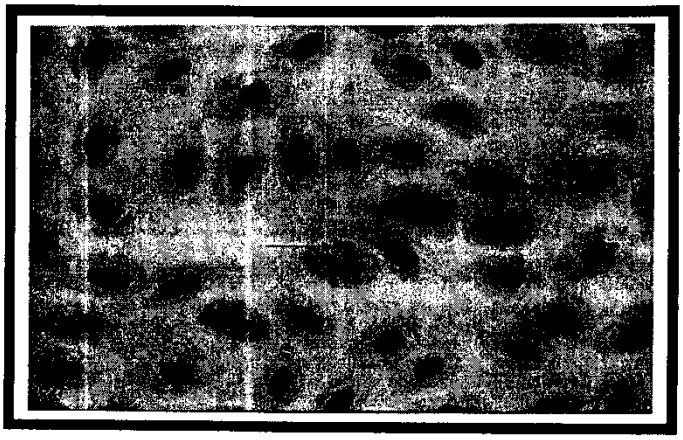

Fig12 : RBC of *C. carpio* (Site 5) show that normal nuclear (⟶), Gemssa stain 400X

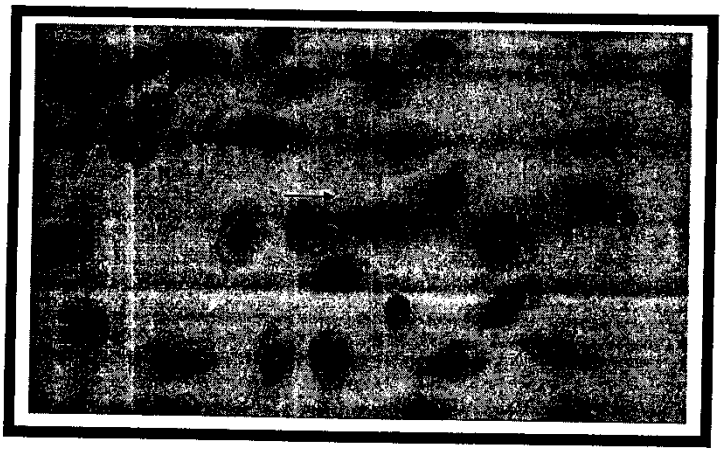

Fig 13: RBC of *C. carpio* (Site 4) Show that micronuclear in some RBC (⟶), Gemssa stain 400X .

Table (1) Concentrations level Na^+ , K^+ and Ca^+ ions in some of fresh water fish which collected from different sites on Shatt-Alarib River .

(P< 0.05) Significant difference

Fish Types	Ions Con.	Stations				
		Site1	Site2	Site3	Site4	Site5
C. carpio	Na^+	50.83±1.42 a	48.77±2.07 ab	48.98±1.24 ab	46.70±1.45 b	56.78±2.00 c
	K^+	91.40±3.88 a	88.70±4.00 ab	85.85±2.43 ab	83.80±3.00 b	97.99±2.4 c
	Ca^{+2}	61.87±3.01 a	62.45±3.21 ab	64.85±3.55 ab	60.48±3.20 a	67.83±2.98 b
L. abo	Na^+	41.80±1.50 a	40.35±2.98 A	38.45±2.60 A	34.4±1.41 b	48.91±3.02 c
	K^+	94.83±2.08 a	92.34±2.79 ab	88.50±2.09 B	83.40±1.81 c	105.4±4.58 d

Table (1) Number of gills chloride cells , RBC micronuclear and respiratory diffuse distance some of fresh water fish which collected from differences sites on Shatt - Alarab River .

(P< 0.05) Significant difference

Fish Types	Parameters	Stations				
		Site1	Site2	Site3	Site4	Site5
C. carpio	Chloride cell of Gilla	1.38±0.08 a	1.41±0.05 a	1.57±0.035 B	1.87±0.085 c	1.35±0.059 a
	Respiratory diffuse distance	13.11±0.73 ab	12.70±0.41 a	14.35±0.15 B	16.71±0.35 c	10.45±0.41 d
	Micronuclear of RBC	4.88±1.70 ab	5.50±1.00 ab	4.38±1.34 A	6.85±0.70 b	1.15±0.90 c
L. abo	Chloride cell of Gilla	1.74±0.098 A	1.85±0.019 a	1.94±0.020 B	2.01±0.034 c	1.61±0.090 d
	Respiratory diffuse distance	14.70±0.86 a	16.85±0.58 a	16.71±0.66 A	15.95±0.45 a	11.85±0.95 b

استخدام بعض التغيرات الفسلجية والنسيجية في غلاصم ودم سمكتي الكارب العادي والخشني كعلامات بايلوجية لتلوث المياه العذبة

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الخلاصـــــة

كجزء أساسي من برنامج بحثي واسع لتقدير تأثير الملوثات المائية على صحة الأسماك في البيئة المائية جاءت فكرت الدراسة الحالية في تحديد العلاقة القائمة بين التعرض المباشر للملوثات البيئية والاستجابات البايلوجيـــة لهـــا ، وأهمية استخدام هذه الاستجابات كعلامات بايلوجية لتلوث المياه العذبة . فقد جمعت عينات من اسماك الكرب العـــادي والخشني من أربعة محطات تقع نسبيا قرب بعض مصادر التلوث الصناعي والبشري المباشر ، بينما اختيرت المحطة الخامسة كسيطرة كونها تقع بعيدا عن مصادر التلوث .

اختبرت العديد من التغيرات النسيجية والفسلجية في غلاصم ودم اسماك التجربة ، وقد دلت النتـــائج وجـــود تغيرات ملحوظة بين المحطات ، وقد أظهرت اسماك المحطتين (٢ ، ٤) التغيرات الأكثر وضوحا مقارنة مع بـــاقي المحطات المدروسة . تمثلت تلك التغيرات نسيجيا بحالات متباينة من فرط التنسج والتضـــخم والنـــزف والالتهابـــات الأرتشاحية ولتنخر وانفصال الطلائية وتكون الأشكال البصلية في نهاية الخيوط الغلصمية ، بينما لوحظ فسلجيا انخفاض في مستوى تركيز ايونات الصوديوم والبوتاسيوم والكالسيوم ، وعدد خلايا الكلورايد بينما سجلت زيادة فـــي ومســـافة الانتشار التنفسي للغلاصم ، إما خلايا الدم الحمر فقد أظهرت زيادة واضحة في معدل عدد النوى الصغيرة للمحطـــات الأربعة مقارنة مع المحطة الخامسة .

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