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The Relation between Bacterial and Heavy Metal Water Pollution and Blood Micronuclei as Biomarkers in the Tigris River Fish

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Abstract:

The objective of this study is to evaluate the bacterial count and heavy metal concentration of river water on fish micronuclei. Fish and water samples are carried out in 1 May to 1 June 2013 from Tigris River. A total of fifty three fish sample are studied. The bacteriological quality of water showed that the total viable count is ranged from 150×10^3 to 352×10^3 cfu/ml and fecal coliform counts was 1250 cell/100ml during the study period. All the metals (Cu, Hg, Pb, and Zn) are within the normal limit, but Cd was slightly elevated in river water samples. The appearance of micronuclei in red blood cells of all fish species is detect , by recording a larger number of it, in (Abu Alsomere , Hishne , Bannini Kaber al fam & Karkoor ahmar) species compared with (Abu AL hakam , Nabbash , Kattan , Himri & Tela shami) species. There is a difference in the percentages of the leukocytes types in different fish species, the highest percentage (12.3) of lymphocyte is recorded in *Barbus xanthopterus* and the lowest (1.5) is in *Garra rufa.*

Key words: Water Quality, Coliform, Heavy Metals, Fish, Micronuclei

Introduction:

"The aquatic environment is the ultimate recipient of an increasing amount of contaminants as a result of the discharge of industrial, agricultural and urban wastes" [1]. The Tigris River is considered to be the main source of water in Baghdad and the change in water quality results in inadequate sanitation and sewerage [2]. Shawaka water area is polluted through municipal waste water, industrial waste water and fisher product [3]. Previous research also, showed the highest bacterial pollution in swim Shawaka area in the Tigris river [4].

A large number of pollutants are responsible for multiple effects on the organisms, including human beings, and ecosystem levels, affecting organ function, reproductive status, species survival, population size and ultimately biodiversity [5]. Fish are excellent subjects for the study of various effects of contaminations present in water

samples since they can metabolize, concentrate, and store waterborne pollutants [6]. Heavy metals concentration in aquatic ecosystems are usually monitored by measuring their concentration in water, sediments and biota [7] which generally exist in low levels in water and attain considerable concentration in sediments and biota [8]. The bacteriological examination of water is of a special significance in pollution studies, as it is a direct measurement of deleterious effect of pollution on human health [9]. Coliform are the major microbial indicator of monitoring water quality[10 , 11].

Hematological parameters are important to evaluate the health condition and physiological status of fish [12, 13] they are also closely related to the response of the fish to the environment, which could exert some influence on their hematological characteristics [14]. Hematologic disorders of fish are marked by aberrations in structure or function of the blood cells (erythrocytes, leukocytes) or the mechanism of coagulation [15]. The micronucleus (MN) test as an index of accumulated genetic damage during the lifespan of the cells is one of the most suitable techniques to identify integrated response to the complex mixture of contaminants [16]. Leucopenia with lymphopenia or a relative neutrophilia is indicative of a stress response or sepsis. In sharks, increases in granulocytes and decreases in lymphocytes can be obtained with bacterial septicemia [17, 18].

Materials and Methods

Fish and Water Samples: Sampling activities (sample/week) are carried out in 1 May to 1 June 2013, at 8:00 am from the shawaka area in the Tigris River in Baghdad city. Freshly caught live healthy fish are collected using conventional fish net traps in the early hours of the day. All the captured fish are placed immediately into a container containing river water. Four water samples are collected from the surface water twice time into sterile 1L glass bottles at 8:00 am and transported to the laboratory in ice box for bacterial analysis within two hours of collection.

Bacteriological Analysis:

The Bacterial Total Viable Count:

The total viable count was done according to the [19] method.

Total Coliform and Fecal Coliform Counts:

The coliform and fecal coliform counts was done according to the [20] method.

Determination of Heavy Metals (Cd, Cu, Pb, and Zn): Heavy metals analysis is done by using an Atomic Absorption Spectrometry NOV-AA350 (Analytic Jena-made in Germany). Mercury is determined by using Hydrate System 55 (Hg HS 55- Analytic Jena).

Micronuclei Test:

Peripheral blood, obtained from each fish and smeared immediately, then the micronuclei test are done according to [21]and [22] methods.

Counting of Different Leukocytes Types:

The different types of leukocytes are determined according to [23] method.

Results and Discussion:

In this study, the microbiological quality of water is elevated. Table 1 shows the total viable count which is ranged from 150×10^3 to 352×10^3 cfu/ml, total coliform (TC) counts are more than 1600 cell/100ml and fecal coliform (FC) counts are 1250 cell/100ml during the study period. This result is higher than the previous local study ranged from 128 to 10000 cfu/ml in four different sites in Tigris River in Baghdad city [4]. Higher bacterial count may be due to new deposition of pollutants.

Table (1): Bacteriological Analysis of River Water

	Total Viable Count (cfu/ml)	Total Coliform (cell/100ml)	Fecal coliform (cell/100ml)
Mean for four sample	245.5×10^3	1600	1250

Results in Table (2) permissible show that all the metals concentration in Tigris River water (Cu, Hg, Pb, and Zn) are within the normal limit, but Cd appears slightly elevated in the river water sample.

Table (2): Metals Concentration in Tigris River Water Samples

	Cd	Cu	Hg	Pb	Zn
Mean for four sample	0.01937	0.0381	Nil	0.00001	0.00667
Standard*	0.005	0.05	0.001	0.05	0.5

***Iraqi water sources standard [24]**

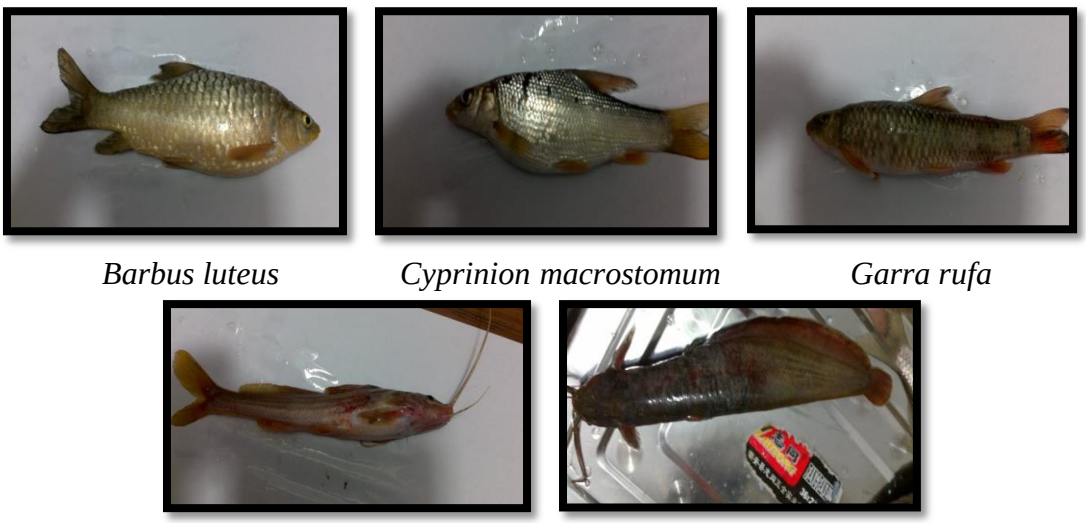
Table (3) shows the average number of micronuclei in nine fish species caught from Shawaka area (Figure 1) and the appearance of micronuclei (Figure 2). The red blood cells of all fish species record a larger number of micronuclei in Abu Alsomere , Hishne , Bannini Kaber al fam and Karkoor ahmar species compared with Abu AL hakam, Nabbash, Kattan, Himri and Tela shami species. This indicates the contamination of the region from which fish were collected during the period of study. The micronuclei test is considered to be a good marker to evaluate the pollution and is supported in several studies. A previous study to investigate pollution in three areas of Baghdad during 2001 showed that these areas were contaminated to varying degrees using micronuclei testing as an indicator to identify the water pollution and their proportions [25]. Also , this technique was adopted in the assessment of three areas of contaminated water from Mariut Lake northern of Alexandria city in Egypt , where the micronuclei appeared in the Tilapia fish taken from a region considered to be a clean region with an increased number in the known contaminated water areas fish [26] . In another study done on several areas located in the Adriatic Sea, the snails *Mytilus galloprovincialis* were collected from several clean areas and transferred to private nets to contaminated areas with ships residues for two days. An increase of chromosomal aberration proportion represented as micronuclei in the snails cells was observed which was an approach to the proportion of chromosomal aberrations in the contaminated area snails. This indicates that the exposure period was sufficient to induce these aberrations [27]. Furthermore, this technology has been adopted as a genetic marker to detect the effect of exposure of *Colossoma macropmum* fish to a certain concentrations of Methylmercury (MeHg) substance[28]. Also, acute and chronic exposure of *Barbus grypus* fish located in laboratory aquarium to low concentrations of Diazinon & Sumicidin pesticides has led to the appearance of micronuclei in red blood cells and increased in number with the increasing concentrations of two pesticides [29] .

Table (3): The Mean of Micronuclei in Erythrocytes of Different Iraqi Fresh Water Fish Species

No.	Common name	Scientific name	Number of fish	Mean of micronuclei ±SD
1	Abu Alsomere	*Mystes pelusus*	7	6 ± 1.0801
2	Abu Alhkam	*Heteropenustes fossilis*	8	5 ± 1.1338
3	Nabbash	*Barbus barbulus*	8	5 ± 0.7559
4	Hishne	*Liza abu*	4	6 ± 1.414
5	Kattan	*Barbus xanthopterus*	6	4 ± 0.6324
6	Bannini kaber al fam	*Cyprinion macrostomum*	4	6 ± 1
7	Himri	*Barbus luteus*	10	4 ± 0.7453
8	Tela shami	*Varicorhinus damascinus*	3	5 ± 0.7071
9	karkoor ahmar	*Garra rufa*	3	6 ± 0.7071
Total samples			53	

The current study show that the pollutants in the study may have caused damage to fish genetic material evidence by recording appearance of micronuclei in red blood cells. The micronuclei in red blood cells of current studied species may be much less than their real number , due to the fact that the fish red blood cell passes five to six divisions since its composition until reaching the blood stream [30] and thus decreasing the number of micronuclei as a result of the repeated divisions [31].

Micronuclei produced by chromosomal changes are considered an indicator of DNA cell damage, so due to the similar molecular structure of genetic material (DNA) in various organisms, any polluting material affect the installation of DNA in particular type of organisms will also affect the genetic material of a different organisms [32]. Moreover, the metabolism of pollutants may converted it to be more toxic within the organism body, these processes in fish are slower compared with mammals , also the toxic influence of pollutants that is observed in fish may be more toxic to mammals including humans [33]. Therefore, pollution of recent water area study does not constitute a threat to fisheries and other aquatic organisms only, but also on humans.



Barbus luteus *Cyprinion macrostomum* *Garra rufa*

Mystes pelusus *Heteropenustes fossilis*

Fig (1): Fish Species

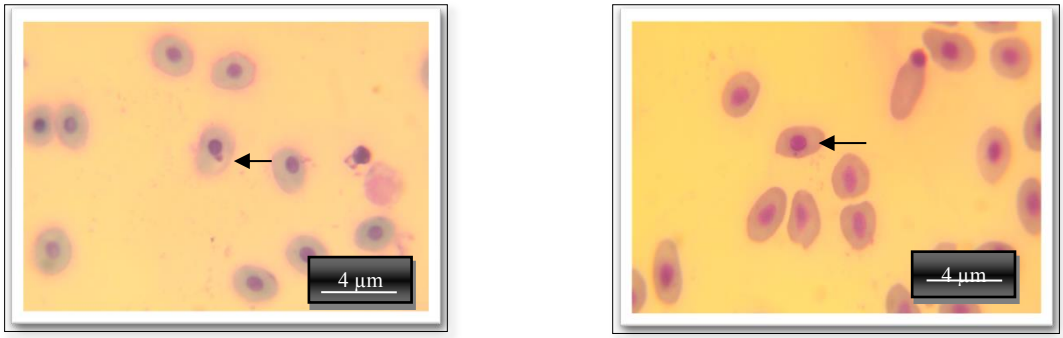


Fig (2): Micronuclei in Red Blood Cells

Table (4) showed the mean of different leukocytes types in nine Iraqi fresh water fish species. In Fig (3) we observed all types of leukocytes and thrombocyte in three fish species (*Liza abu, Barbus xanthopterus* and *Cyprinion macrostomum*), while the basophils are absent in five fish species (*Mystes pelusus, Heteropenustes fossilis, Barbus luteus, Varicorhinus damascinus* and *Garra rufa*), also we didn't observe the monocytes in *Barbus barbulus* . There is a difference in the percentages of the leukocytes types in different fish species, generally the lymphocytes percentage is the highest among the types of leukocytes varied according to fish species, whereas the highest percentage (12.3%) of lymphocyte recorded in *Barbus xanthopterus* and the lowest (1.5%) in *Garra rufa* and its percentages are convergent (6.8, 6.5, 6%) in *Liza abu, Barbus barbulus* and *Cyprinion macrostomum*, (2.9, 2.7%) in *Mystes pelusus* and *Heteropenustes fossilis*, (4.6, 3.6%) in *Barbus luteus* and *Varicorhinus damascinus* respectively. It has been previously reported that higher lymphocytes count was found in fish from production systems with marginal water quality [13]. Our study showed the percentages of lymphocytes are variable among fish species, where the high percentage recorded in *Barbus xanthopterus* then (*Liza abu, Barbus barbulus, Cyprinion macrostomum*) followed by (*Barbus luteus, Varicorhinus damascinus, Garra rufa*) respectively .

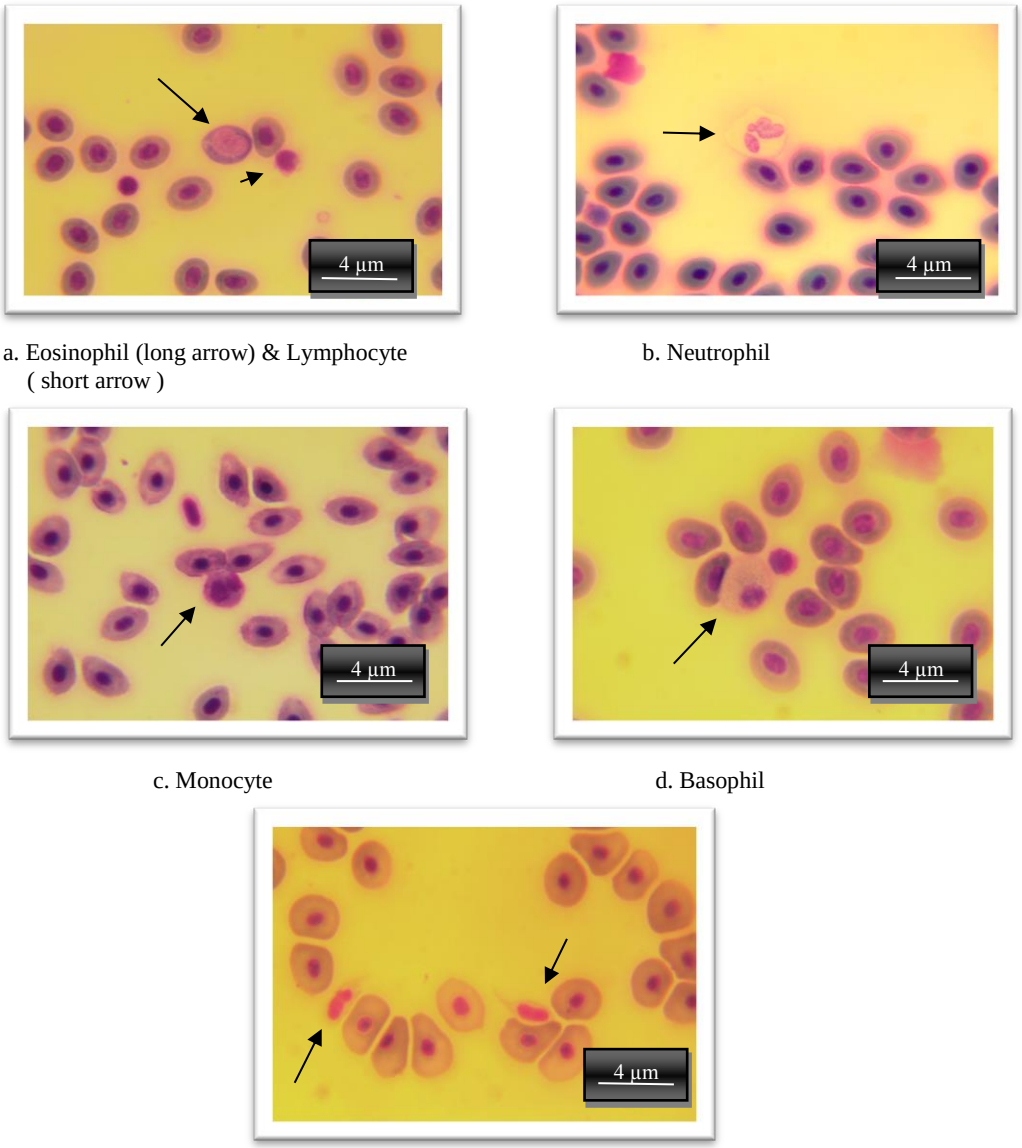
The neutrophil comes second after lymphocyte in the proportion of its appearance in blood smears, where the highest percentage (8.4%) observed in *Garra rufa* and the lowest (2.5%) in *Heteropenustes fossilis* . Then comes the monocyte, where the highest percentage (4.6%) is recorded in *Garra rufa* also and the lowest (0.4%) in *Cyprinion macrostomum* fish. It was mentioned that the high proportion of neutrophil associated with occurrence of inflammatory processes, also increasing the proportion of monocyte occurs as a response to the existence of inflammation in teleost fish [34, 35]. As for the eosinophil, has the highest percentage (3.4%) in *Barbus barbulus*, the lowest (0.6%) in *Barbus luteus*. Some reports indicate that fish eosinophils are involved in inflammatory responses but have limited phagocytic capability [33,35]. Generally, the eosinophils are less common in teleost's whereas basophils are rare [17] and this is observed in the current study.

The thrombocyte shows a high proportion (7.2%) in *Mystes pelusus* and *Barbus xanthopterus*, a low proportion (1.6%) in *Garra rufa*. It is known that the thrombocyte play a large role in mediating the clotting response , and they are also responsible for controlling fluid loss from surface wounds in fish [18] .

In the current, study the *Trypanosoma* parasite observed in one blood smear of *Mystes pelusus* fish (Fig 4) where this organism is the most common parasite in infected fish blood [15].

Table (4): The Mean of Leukocytes Types in Nine Iraqi Fresh Water Fish Species

Catch fish	Lymphocyte	Eosinophil	Neutrophil	Basophil	Monocyte	Thrombocyte
Mystes pelusus	2.9 ± 0.345	2.7 ± 1.061	2.6 ± 0.435	0	0.9 ± 0.281	7.2 ± 0.499
Heteropenustes fossilis	2.7 ± 0.297	2.4 ± 0.388	2.5 ± 0.531	0	0.8 ± 0.231	4.9 ± 0.458
Barbus barbulus	6.5 ± 0.366	3.4 ± 0.253	5.3 ± 0.301	0.8 ± 0.198	0	3.7 ± 0.543
Liza abu	6.8 ± 0.645	1.2 ± 0.573	2.7 ± 0.991	0.9 ± 0.331	0.7 ± 0.129	2.7 ± 0.660
Barbus xanthopterus	12.3 ± 0.402	0.9 ± 0.432	3.8 ± 0.432	0.4 ± 0.116	0.8 ± 0.242	7.2 ± 0.549
Cyprinion macrostomum	6 ± 0.330	1 ± 0.095	4.4 ± 0.311	0.3 ± 0.171	0.4 ± 0.129	1.9 ± 0.853
Barbus luteus	4.6 ± 0.499	0.6 ± 0.126	3.5 ± 0.329	0	0.6 ± 0.103	6 ± 0.257
Varicorhinus damascinus	3.6 ± 0.757	0.9 ± 0.2	3.6 ± 0.702	0	1.1 ± 0.208	5.7 ± 0.321
Garra rufa	1.5 ± 0.305	1.3 ± 0.173	8.4 ± 0.305	0	4.6 ± 0.404	1.6 ± 0.378



a. Eosinophil (long arrow) & Lymphocyte (short arrow)

b. Neutrophil

c. Monocyte

d. Basophil

e. Thrombocyte

Fig (3): The Five Types of Leukocytes with Thrombocyte in Blood Smear of Different Fish Species

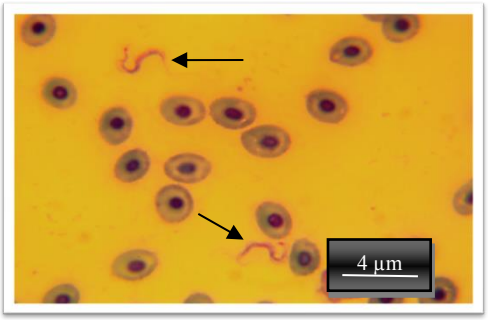


Fig (4): The *Trypanosoma* Parasite in *Mystes pelusus* Blood Smear

Conclusion:

The recent study shows that there is a clear interaction between micronuclei test for fish species and the level of total coliform and fecal coliform counts of water samples. On the other hand, the normal metals level may not be related with micronuclei aberrations in fish species under study. The highest percentage (12.3%) of lymphocyte is recorded in *Barbus xanthopterus* and the lowest (1.5 %) in *Garra rufa*.

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العلاقة بين التلوث المائي بالعناصر الثقيلة والبكتريا والانوية الصغيرة في الدم كمؤشرات حيوية في أسماك نهر دجلة

اسراء عبد الجبار إبراهيم **أسماء سامي إبراهيم الخياط**

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الخلاصة:

تهدف الدراسة الحالية الى التحري عن تأثير العدد البكتيري وتركيز العناصر الثقيلة لمياه النهر على الانوية الصغيرة للأسماك. جمعت الأسماك وعينات المياه من نهر دجلة بتاريخ 1 أيار الى 1حزيران 2013. تم دراسة ما مجموعه ثلاثة وخمسون عينة من الأسماك. أظهرت دراسة النوعية البكتريولوجية للمياه بأن التعداد العدد الكلي الحي تراوح بين 150×10^3 الى 352×10^3 ml / cfu وعدد البكتريا القولونية البرازية 1250 خلية /100mL خلال فترة الدراسة. جميع العناصر الثقيلة (Cu ، Hg ، Pb و Zn) كانت ضمن الحد الطبيعي باستثناء عنصر الكادميوم Cd، اذ ارتفعت نسبته قليلا ضمن عينات ماء النهر. لوحظ ظهور الانوية الصغيرة في خلايا الدم الحمر لجميع أنواع الأسماك مع تسجيل عدد أكبر منها في اسماك (أبو الزمير، خشني، بنيني كبير الفم وكركور احمر) مقارنة بأسماك (أبو الحكم، نباش، قطان، حمري وتيلة دمشقية). كان هناك اختلاف في نسب أنواع خلايا الدم البيض في الأنواع المختلفة من الأسماك، اذ سجلت اعلى نسبة من الخلايا اللمفية (12.3) في سمكة *Barbus xanthopterus* واقل نسبة منها في سمكة *Garra rufa* .

الكلمات المفتاحية: نوعية المياه، القولونية، عناصر ثقيلة، اسماك، انوية صغيرة.