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Histopathology and micronuclei induction as pollution biomarkers in common carp, *Cyprinus carpio* from southern Iraq

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Abstract

This study aimed to assess the histopathological alterations and micronuclei induction in the gill, kidney and liver of common carp, *Cyprinus carpio* from Al-Huwaizah Marshes, Al-Hammar Marshes and Al-Sweap River. Thirty *C. carpio* were sampled at each locality using monofilament gillnets. Most prominent histopathological alterations recorded in the gill included formation venous sinus in primary lamellae, oedema, curling and distortion of primary lamellae fusion of secondary lamellae, hyperplasia of epithelial cells, necrosis, curling of secondary lamellae, severe dilation of lamellar capillaries, clavate lamellae formation at the tip of secondary lamellae, a proliferation of cartilage tissue in primary lamellae and lifting of the lamellar epithelium whereas congestion, fibrosis with proliferative inflammation, necrosis area, degenerated cells, vacuolar degeneration, vacuolation, nuclear pyknosis, hypertrophy of hepatocytes were reported in the liver. In the kidney, the most pronounced changes were necrosis of the hematopoietic tissue, hyaline droplet and activation of melanomacrophage centres. A higher prevalence of alterations was observed in the population from Al-Huwaizah Marshes, however, Al-Hammar population exhibited severe pathologies. Micronuclei frequency was significantly higher at Al-Huwaizah Marsh ($p < 0.05$) with low frequency being recorded at Al-Sweap River. This study attests to the effectiveness of histopathology and micronucleus induction as potential tools for discriminating the health of populations from different water bodies.

Keywords: Histopathology; micronuclei induction; *Cyprinus carpio*; pollutants

1. Introduction

Aquatic ecosystems are known to provide sanctuary to biodiversity yet are susceptible to anthropogenic activities such as pollution (Angeler et al., 2014). Al-Huwaizah Marsh and Al-Sweap River in southern Iraq had been receiving agricultural drainwater from Iran since late 2010 until early 2011 (Stevens & Ahmed, 2011). This agricultural drainwater caused significant fish and invertebrate mortality. Although the chemical properties of the drainwater were not determined, it is suspected that either elevated levels of pesticides and/or metals in the drainwater may have contributed to the fish and invertebrate mortality. However, it should also be noted that the salinity of the drainwater from Iran was approximately 16 ‰, whereas the salinity of the marshes was 1-1.5 ‰; therefore, salinity may have also contributed to fish and invertebrate mortality (Naser, unpublished data). Salman (2011) showed that the southern Iraqi marshes are moderately contaminated with hydrocarbons and heavy metals, whereas recent studies reported that marshes in southern Iraq are subjected to high levels of pesticides of different formula (Yasser et al., 2008; Naser, 2009; Yasser & Naser, 2011; Al-Ali 2012; Yasser, 2012, Al-Gheezy et al., 2018, Yasser & Naser, 2023).

In the present study, we investigated histological alterations, taking into account that histological changes have long been recognized as reliable biomarkers of contaminant exposure in fish (Bernet et al., 1999; Van der Oost et al., 2003; Zimmerli et al., 2007). In general, chemical toxicity depends not only on the total chemical concentration in the environment but also on how readily the fish can absorb these different chemicals (Di Giulio & Hinton, 2008). The exposure of fish to chemical contaminants is likely to induce several lesions in different organs such as the gill (Paul & Banerjee, 1997), kidney (Cengiz, 2006; Lukin et al., 2011), and liver (Abiona et al., 2019). Furthermore, it has been indicated that histopathology could be a biomarker of the general health of fish and is considered as a mirror that reflects the exposure to a variety of anthropogenic pollutants (Van der Oost et al., 2003). Complementing histopathology could be micronuclei induction which has also been shown to be sensitive to genotoxic agents (Da Rocha et al., 2009).

Over the last few decades, the use of fish as appropriate models for genetic monitoring of toxic chemicals in aquatic environments has become popular. In particular, micronuclei (MN) are induced in fish exposed to genotoxic substances under both laboratory and field conditions (Al-Sabti & Metcalfe, 1995; Cavas et al., 2005). The micronucleus test on fish has played an important role in assessing exposure to water pollutants and has proved to provide an early warning of genotoxic threats to fish, their ecosystem, and finally humans (Al-Sabti & Metcalfe, 1995; Hayashi et al., 1998; Al-Sabti, 2000). Induction of cytogenetic damages in fish could help monitor not only selected genotoxic agents in the laboratory but also the presence of genotoxicants in surface waters and different freshwater ecosystems (Bhatnagar et al., 2016; Hussain et al., 2018). Various studies have found that the peripheral erythrocytes of fish have a high incidence of micronuclei after exposure to different pollutants under field and laboratory conditions (Cavas et al., 2005; de Lemos et al., 2007; Melo et al., 2014).

Cyprinus carpio was introduced in 1980 in Iraqi water bodies and is now widely distributed across the country. As bottom-dwelling freshwater fish, their feeding habits expose them to many different types of environmental contaminants. It is an omnivorous, and widely used as a sentinel organism in biomonitoring programs and toxicity bioassays (Van der Oost et al., 1998; Chuiko, 2000; de la Torre et al., 2002) due to their role in the biotic communities and is easy to capture (Falfushynska & Stolyar, 2009). *Cyprinus carpio* has now been proposed as a suitable organism for genotoxicity and mutagenicity studies (Pandrangi et al., 1995; Nepomuceno et al., 1997). Therefore, the present study aimed to assess the histopathological alterations and micronuclei induction in the gill, kidney and liver of common carp, Cyprinus carpio from Al-Huwaizah Marshes, Al-Hammar Marshes and Al-Sweap River in southern Iraq. It was hypothesised that histopathological condition and micronuclei frequency will differ between the three water bodies.

2. Materials and methods

Study Area

The study was conducted in southern Iraq in the two marshes, Al-Huwaizah (31°28'16.24"N; 47°34'30.81"E) and Al-Hammar (30°46'51.61"N 47°; 2'32.04"E), and the Al-Sweap River (30°58'18.34"N; 47°30'15.93"E) (Fig. 1). Al-Hammar marsh receives water from the transboundary Euphrates river, which drains a catchment characterised by many military factories (Al-Shawi, 2006; Hassan et al., 2010). Al-Huwaizah Marshes is fed by the Tigris River which originates from the mountains of the Eastern Anatolia of Turkey. The Tigris

River catchment is characterised by metallurgic industries such as Ergani Copper Plant and military industries (Karadede-Akin & Ünlü, 2007; Varol, 2011). Al-Sweap River is a 35 km long canal draining water from the Al-Huwaizah Marshes and feeding the Shatt Al-Arab River (Ajeel & Abbas, 2012).

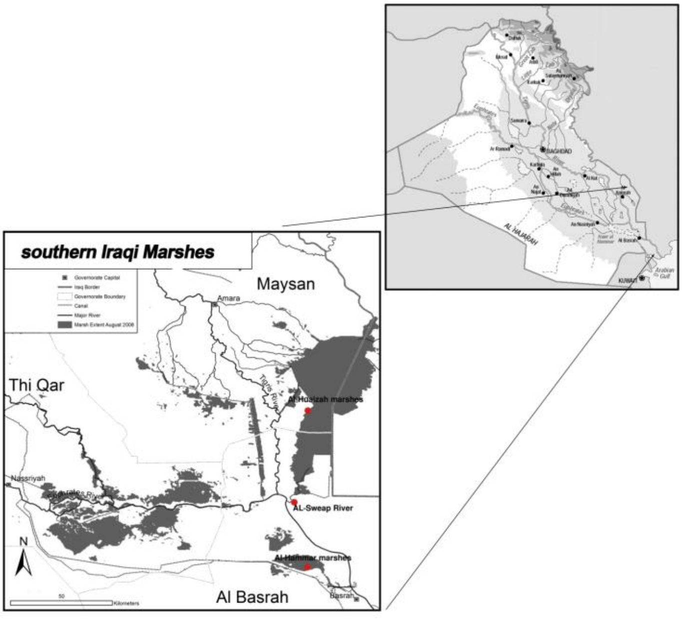

Figure 1. Southern Iraqi marshes with three sampling sites.

Fish sampling and processing

Thirty (30) *C. carpio* were sampled from each locality using monofilament gill nets. Fish were kept in holding tanks filled with water from marshes and transported alive to the field laboratory. In the field laboratory, fish were weighed and the length was measured. To minimise variation due to age and weight, fish of the same weight and length were preferred for histological examination.

Tissue preparation for histological examination

The gills, kidney and liver of each fish were removed immediately after euthanasia to overcome autolysis and samples were fixed in Bouin's solution (Roberts, 2012). The tissues were dehydrated in graded alcohol solutions of 50 %, 70 %, 95 % and 100 %. Tissues were cleared twice in xylene and embedded in paraffin. Paraffin sections of tissues were cut into 5-6 μm thicknesses and stained with hematoxylin and eosin and then photographed and examined with the aid of light microscopy.

Micronucleus frequency test

Micronuclei are round or oval, non-refractile and with no connection to the main nuclei, and the diameter is less than one-third of the main nuclei diameter. Peripheral blood samples were drawn from the caudal vein of the specimens and smeared on clean slides. Peripheral blood smears were fixed in methanol for 10 min and left to air-dry at room temperature and stained with 5 % Giemsa in Sorenson buffer (pH 6.9) for 20 min. Replicate slides per specimen were prepared for every sampling time. For micronuclei analysis, a total of 1000 erythrocytes were examined for each specimen under the light microscope. Micronuclei identification was carried out following Fenech et al. (2003) protocol. The frequency was calculated as follows:

$$\text{MN \%} = \frac{\text{Number of cells counted}}{\text{Total number of cell counted}} \text{ x } 100$$

Statistical analysis

To test significant differences among histopathological alterations depending on site collection the chi-square test ($p < 0.01$ and $p < 0.05$) was used. Also, to explore significant differences in mean values of micronuclei alterations of fish depending on sampling sites ANOVA test was performed. Chi-square test and ANOVA test were conducted using SPSS (SPSS 16.0 version software for Windows).

3. Results

Histopathological responses in the gill

Gill showed signs of proliferative alterations which were characterized by hyperplasia and necrosis at the three sites (Figs. 2, 3, Table 1). Degenerative alterations such as the formation of venous sinus, lifting of lamellae epithelium, fusion of secondary lamellae, curling of secondary lamellae, dilation of lamellar capillaries, clavate lamellae, atrophy of secondary gills lamellae, proliferation of cartilage tissue in primary lamellae, desquamation and disruption of cartilaginous core, oedema, loss of secondary lamellae and curling with distortion of primary lamellae were prominent at the three sites with population from Al-Huwaizah Marshes showing higher prevalence followed by AL-Hammar Marshes and Al-Sweap River, respectively (Table 1).

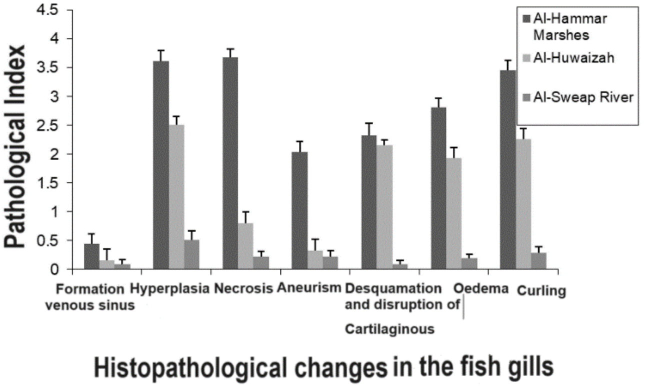

Figure 2. Pathological index observed in the gills of *C. carpio* from three southern Iraqi marshes in May 2011.

However, the severity of pathologies was higher in the AL-Hammar Marshes followed by Al-Huwaizah Marches and Al-Sweap River, respectively (Fig. 2). The prevalence of histopathological in the gills has shown a significant difference between the Al-Sweap River and the two other marshes ($p < 0.05$).

Table 1. The prevalence (%) of several histopathological alterations observed in common carp, *Cyprinus carpio* sampled from the three southern Iraqi marshes. Significant values are labelled with Bold. NS: non-significant.

Histopathological alterations	AL-Huwaizah Marshes (N=30)	AL-Hammar Marshes (N=30)	AL-Sweap River (N=30)	*P*-Value
Gill pathologies				
Formation of venous sinus	43.33	26.66	20	**< 0.01**
Fusion of secondary lamellae	56.66	30	16.66	**< 0.01**
Hyperplasia	26.66	50	13.33	**< 0.01**
Necrosis	56.66	30	6.66	**< 0.01**
Curling of secondary lamellae	50	26.66	20	**< 0.01**
Dilation of lamellar capillaries	30	16.66	3.33	**< 0.01**
Clavate lamellae	33.33	16.66	6.66	**< 0.01**
Atrophy of secondary gill lamellae	26.66	20	10	**< 0.05**
Proliferation of cartilage tissue in primary lamellae	53.33	26.66	16.6	**< 0.01**
Desquamation and disruption of cartilaginous core	26.66	20	3.33	**< 0.01**
Lifting of lamellar epithelium	26.66	13.33	3.33	**< 0.01**
Oedema	43.33	26.66	3.33	**< 0.01**
Loss of secondary lamellae	23.33	10	0	**< 0.05**

Curling and distortion of primary lamellae	50	13.33	10	**< 0.01**
Liver pathologies				
Congestion	46.66	16.66	3.33	**< 0.01**
Fibrosis	20	10	0	NS
Necrosis	23.33	40	3.33	**< 0.01**
Degenerated cells pycnosis	36.66	16.66	10	**< 0.01**
Nuclear degeneration	46.66	23.33	6.66	**< 0.01**
Vacuolation	30	16.6	6.66	**< 0.01**
Hypertrophy of hepatocytes	23.33	13.3	10	**< 0.05**
Kidney pathologies				
Destruction of the glomerulus	20	10	3.33	**< 0.01**
Expansion of space in Bowman's capsule	23.33	6.66	3.33	**< 0.01**
Tubule destruction	3.33	3.33	3.33	NS
Pycnotic nuclei in the hematopoietic epithelial cell	40	20	6.66	**< 0.01**
Hyaline droplets	43.33	26.66	10	**< 0.01**
Inflammation with hematopoietic tissue fibrosis	13.33	3.33	0	**< 0.01**
Activation of the melanomacrophages centers	23.33	10	0	**< 0.01**
Occlusion of the tubular lumen	20	10	3.33	**< 0.05**
Necrosis	43.33	26.66	10	**< 0.01**
Cell hypertrophy with narrowing tubular lumen	16.66	6.66	3.33	**< 0.01**
Haemorrhage	16.66	36.66	13.33	**< 0.01**

Histopathological findings in the liver

Histopathological alterations reported in the liver include fibrosis, degenerated cells, vascular degeneration, nuclear pyknosis, vacuolation and hypertrophy of hepatocytes (Table 1, Figs. 4, 5). However, there was a significant difference in the prevalence and severity of these alterations between Al-Sweap River and the two marshes ($p < 0.05$). Histopathological alterations were most prominent in fish populations from Al-Huwaizah Marshes, followed by Al-Hammar Marshes and Al-Sweap River, respectively (Table 1). However, Al-Hammer Marshes exhibited a higher pathological index relative to the two other marshes (Fig. 4).

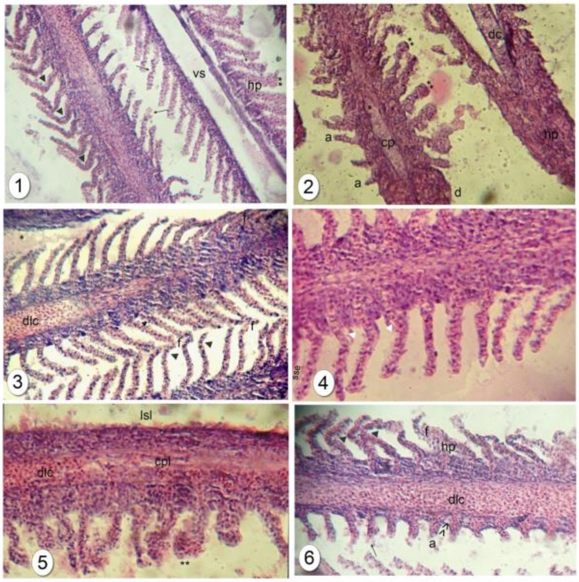

Figure 3. Gill histopathology: Decrease in the length of primary lamellae (*d*; 2); formation venous sinus in primary lamellae (*vs*; 1); fusion of secondary lamellae (*f*; 3, 6); hyperplasia of epithelial cells (hp;1, 2, 6); necrosis and desquamation (➞ *black arrows*; 1, 6); curling of secondary lamellae (▶ *black head arrows*; 1, 3, 6); sever dilation of lamellar capillaries (*dlc*; 3, 5, 6); clavate lamellae formation at the tip of secondary lamellae (**; 1, 2, 5); atrophy of secondary gills lamellae (*a*; 2, 6); proliferation of cartilage tissue in primary lamellae (*cp*; 2); desquamation and disruption of cartilaginous core of primary lamellae (*dc*; 2); separation of lamellar epithelium (*sse*; 4); epithelial separation and lifting base of secondary lamellae showing oedematous condition (*white head arrows*; 4); loss of secondary lamellae (*lsl*; 5); curling and distortion of primary lamellae (*cpl*; 5) hypertrophied of chloride cells (double head arrows, 6); vs venous sinus, *hp* hyperplasia, necrosis, curling lamellae, *dlc* dilation lamellae capillaries, ** clavate lamellae, *cp* cartilage proliferation, *dc* desquamation cartilaginous lamellae, *sse* separation lamellae, *white head arrows* oedema, *lsl* loss secondary lamellae, >> hyper trophied chloride cells, 1-6 hematoxylin-eosin, I PAS. 2, 3, 4, 5, 6; × 40, 1; × 10.

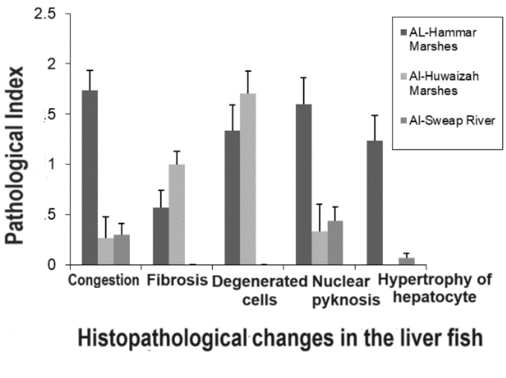

Figure 4. Pathological index observed in the liver of *C. carpio* from three southern Iraqi marshes in May 2011.

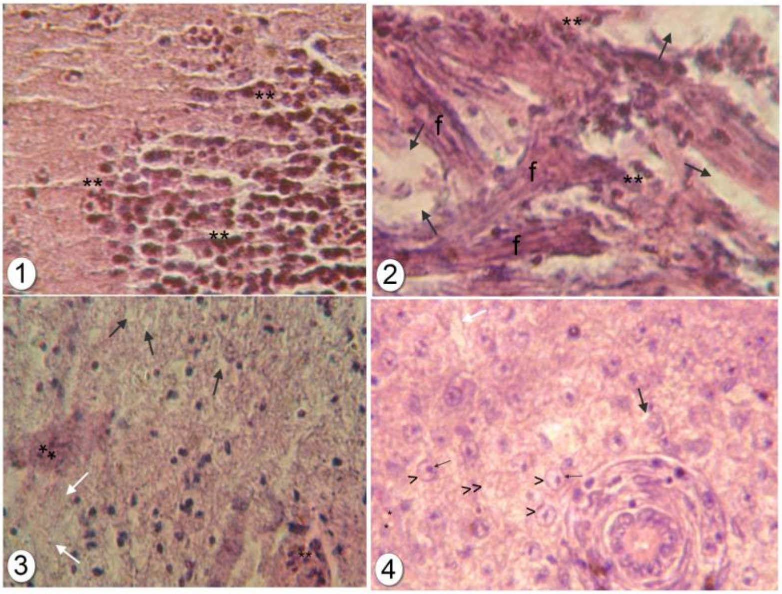

Figure 5. Liver histopathology: Asterisks indicate areas of congestion (**; 1, 2, 3); fibrosis with proliferative inflammation (*f*; 2); necrosis area (➡ *thick black arrows*; 2, 3, 4); degenerated cells (*white arrows*; 3); vascular degeneration (>> *double head arrows*; 4); vacuolation (*; 4); nuclear pyknosis (⟶ *thin black arrows*; 4); hypertrophy of hepatocytes (> *head arrows*; 4); ** congestion, f fibrosis, necrosis, degenerated , >> vascular,* vacuolation, ⟹ Nuclear pyknosis, > hypertrophy.

Histopathological findings in the kidney

Histopathological alterations in the kidney were dominated by circulatory disturbances which included destruction of the glomerulus, expansion of space inside the Bowman's capsule, tubule destruction, pyknotic nuclei in the epithelial cell of hematopoietic tissue, haemorrhage, hyaline droplet, inflammation with fibrosis of the hematopoietic tissue, activation of the melanomacrophage centres (MMCs), occlusion of the tubular lumen, necrosis, cell hypertrophy and narrowing tubular lumen (Fisg. 6, 7,Table 1). These alterations have shown a significant difference between Al-Sweap River and the two marshes ($p < 0.05$). The alterations were prominent at Al-Huwaizah Marshes, followed by AL-Hammar Marshes and Al-Sweap River, respectively (Table 1).

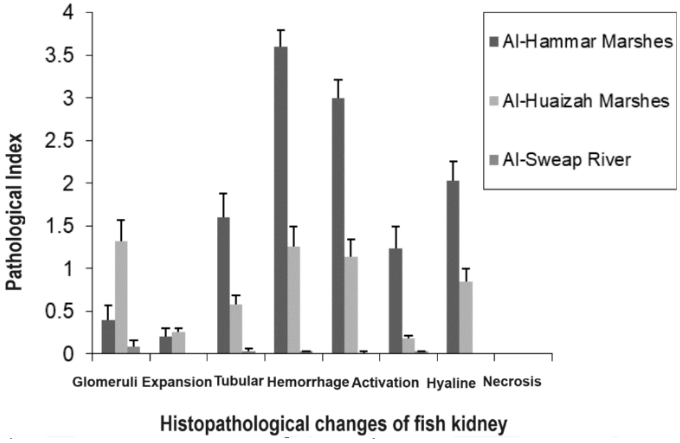

Figure 6. Pathological index observed in the kidney of *C. carpio* from three southern Iraqi marshes in May 2011.

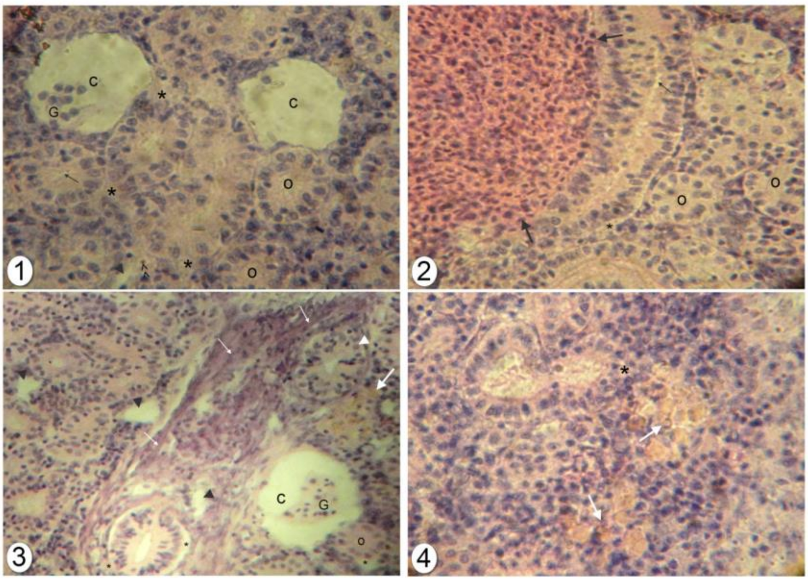

Figure 7. Kidney histopathological sections of common carp, *Cyprinus carpio* from the south of Iraq. Destruction of the glomerulus (*letter G*; 1, 3); expansion of space inside the Bowman's capsule (*letter C*; 1, 3); the asterisks indicate areas of tubule destruction (*; 1, 2, 3, 4); necrosis of the hematopoietic tissue (▶ *Black head arrows*; 1, 2, 3); pycnotic nuclei in the epithelial cell of hematopoietic tissue (>>; 1); hypertrophy cells and narrowing of the tubular lumen (→ *thin black arrow*; 1, 2);occlusion of tubular lumen (*letter O;* 1, 2, 3); haemorrhage (⟶ *thick black arrows*; 2); hyaline droplet (*white head arrow*; 3); proliferative inflammation with fibrosis of the hematopoietic tissue (*thin white arrows*, 3); activation of the melanomacrophage centres (*thick white arrows*; 3, 4); *G* glomerulus contraction, *c* space inside Bowman's, tubule destruction, haemorrhage, necrosis, >> pycnotic, hypertrophy cells with narrowing tubular lumen, *o* occlusion tubular lumen, *white head arrow* hyaline droplet, *thick white arrow* melanomacrophage, 1-4 hematoxylin- eosin, I PAS, 1,2,3,4; × 40

Micronucleus test data

The obtained results are summarised in Figure 8. Generally, clastogenic effect represented by the formation of micronucleus has increased in the following rank: Al- Huwaizah Marsh > Al-Hammar Marshes > Al-Sweap River. The high MN frequency was recorded in fish from Al-Huaizah Marshes (5.7 %) followed by Al-Hammar (3.5 %) and Al-Sweap River (2.2 %), respectively. There was an apparent significant increase in MN frequency in the blood cells of carp from the three sites ($p < 0.05$).

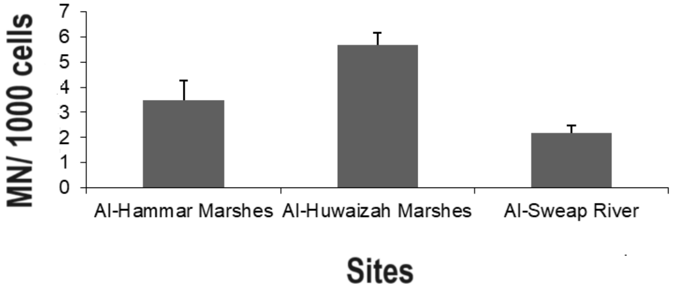

Figure 8. Micronuclei percentage frequency in *C. carpio* captured from three southern Iraqi marshes in May 2011.

4. Discussion

The Euphrates and Tigris rivers are transboundary systems which are feeding Al-Hammar and Al-Huwaizah marshes, respectively. The water quality of these two marshes has been extensively studied (Al-Saad et al., 2010; Hassan et al., 2010; Al-Musawi et al., 2018; Hasab et al., 2020). The marshes are frequently experiencing uncontrolled flooding that adversely affects the water quality due to the seepage of contaminants from metallurgic industries, mines and military ordnance (Richardson & Hussain, 2006; Hassan et al., 2010). Moreover, overfishing through the use of pesticides and their extensive application in agriculture have adversely impacted the water quality in these marshes (Naser, 2009; Al-Gheezy et al., 2018; Yasser & Naser, 2023). Both marshes receive water from polluted river systems, however, Al-Hammar marshes are shown to be more polluted than the Al-Huwaizah marshes. Salman (2011) reported higher concentrations of trace metals and hydrocarbons at the Al-Hammar marshesthan at the Al-Huwaizah marshes. Moreover, Awad et al. (2008) reported substantially higher trace metal concentrations in the sediment from Al-Hammar marshes than at the Al-Huwaizah marshes.

Fish gills are one of the most sensitive and vulnerable tissues of the teleost fish because they are located externally and are in intimate contact with the external water environment (Roberts, 2012). Due to their large surface area, fish gills are sensitive to even mild pollution of the surrounding water environment (Roberts, 2012; Strzyzewska et al., 2016). In the present study, most gill alterations showed higher prevalence at the Al-Huwaizah marshes, however, the severity of pathologies was higher in the fish population from Al-Huwaizah marshes, followed by that from Al-Hammar marshes, while Al-Sweap River population exhibiting less severity. Common alterations included, lifting of lamellae epithelium, hyperplasia of epithelial cells, hypertrophy of chloride cells, oedema, necrosis, formation venous sinus, fusion of secondary lamellae, curling of secondary lamellae, dilation of lamellar capillaries, clavate lamellae, atrophy of secondary gills lamellae, proliferation of cartilage tissue in primary lamellae (Table 1). These findings are comparable to those reported in other studies across the globe (Baiomy, 2016; Javed et al., 2017; Abiona et al., 2019; Lebepe et al., 2020).

Gill is a primary site for respirations and is covered by a sensitive epithelium which is the site of exchange of gases, ionic regulation, acid-base balance and nitrogenous waste excretion (Roberts 2012; Javed et al., 2017). Due to their location and the crucial functions they perform in fish, gills become the first site to be affected by waterborne contaminants. Abdel-Moneim et al. (2012) reported vasodilation, lamellar fusion, hyperplasia, epithelial and lamellar capillary aneurysms, and epithelial lifting in heavy metal-contaminated wetlands. Moreover, vasodilation and oedema of epithelium, lamellar aneurysm, proliferation of filamentary epithelium and lamellae fusion were observed in fish exposed to copper (Velcheva et al., 2013). Georgieva et al. (2014) reported epithelium lifting, oedema, proliferation of the stratified epithelium and glandular cells, and lamellae fusion in the gill of fish exposed to pesticides, thiamethoxam.

Most histopathological alterations such as epithelial lifting, hypertrophy, hyperplasia and oedema are deemed to be adaptations to new conditions to protect against excessive penetration of contaminants from the external water environment to the blood vessels in the gills (Baiomy, 2016; Strzyzewska et al., 2016). Although histopathological alterations in the gills could also be associated with physical injuries in aquatic ecosystems (Lukin et al., 2011), the pollution effect in fish from the Al-Huwaizah and Al-Hammar marshes cannot be dismissed.

The liver is the primary target organ for xenobiotic compounds, as its main role includes uptake, biotransformation and excretion of contaminants (Ameur et al., 2012; Roberts, 2012). However, exposure to elevated concentrations was found to cause oxidative stress which consequently resulted in tissue injuries (Ameur et al., 2012). In the present study, the liver of *C. carpio* exhibited nuclear pyknosis, fibrosis, vacuolation, degenerated cells, vascular degeneration and hypertrophy of hepatocytes. These findings corroborate those observed in fish exposed to different contaminants i.e. waterborne metal(loid)s (Figueiredo-Fernandes et al., 2007; Anjum et al., 2014; Javed et al., 2017), crude oil and dispersed oil (Agamy 2012), pesticides (Korkmaz et al., 2009), and phenol and phenolic compounds (Abdel-Hameid 2007).

In fish livers, histopathological alterations may occur as a result of a complex interaction of pollution stress and cell components (Pal et al., 2011). Thus, hepatic changes suggest a mobilization of defensive mechanisms in an endeavour to detoxify pollutants. In the present study, hepatocytes hypertrophy, congestion and nuclear pyknosis showed high severity at Al-Hammar marshes. Hepatocellular hypertrophy is described as an adaptive response to increase detoxification capacity of the liver (Salamat & Zarie, 2012), whereas congestion probably indicates increased blood volume flowing into the liver to detoxify the organism (Jabeen et al., 2018). The vacuolization of hepatocytes is associated with an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the systemic circulation (Jiraungkoorskul et al., 2002). These alterations were also observed under the exposure of metal(loid)s (Anjum et al., 2014; Javed et al., 2017) and organic compounds (Abdel-Hameid, 2007).

The kidney is one of the first organs to be affected by contaminants as they are responsible for osmoregulation. It metabolises ammonia, urea and creatinine then excrete nitrogen-containing waste products (Cengiz, 2006; Camargo & Martinez, 2007). Common alterations reported in the three marshes include destruction of the glomerulus, expansion of space inside the Bowman's capsule, tubule destruction, pyknotic nuclei in the epithelial cell of hematopoietic tissue, haemorrhage, hyaline droplet, occlusion of the tubular lumen, necrosis, cell hypertrophy and narrowing tubular lumen with most alterations exhibiting higher severity at Al-Hammar marshes, followed by Al-Huwaizah marshes and Al-Sweap River, respectively. These findings are comparable to those observed in fish exposed to municipal wastes (Kaur & Dua, 2016), deltamethrin insecticides (Cengiz, 2006) and aluminium (Hadi & Alwan, 2012).

Moreover, Velma & Tchounwou (2010) reported progressive dilation of tubules, tubular necrosis, renal tubular separation, degeneration of hematopoietic tissue and tubular lumen in fish exposed to chromium. Hyperplasia of MMCs and tubular degeneration was observed in fish exposed to herbicides (Salazar-Lugo et al., 2011), whereas MMCs, oedema, lymphocytic infiltration foci, granulomas, dilation of glomerular capillaries and glomerulo nephritis, degeneration of Bowman's capsule and shrunken glomerular tuft were observed in an oil-contaminated water body (Lukin et al., 2011). Melanomacrophages proliferation and inflammation with hematopoietic tissue fibrosis were only observed at the Al-Hammar and Al-Huwaizah marshes, which may be associated with contaminants in these two marshes. According to Kaur & Dua (2016), MMCs are always associated with necrosis and they show defence mechanisms against contaminants entering the fish body.

Micronuclei test is one of the most popular and promising tests of environmental genotoxicity and has served as an index of cytogenetic damage for over three decades (Fenech et al., 2003, Galindo & Moreira, 2009). Micronucleus analysis has been used to assess mutagen compounds' effect in various environments as it is relatively simple, reliable and sensitive (Galindo & Moreira, 2009). Increased MN has been observed if fish are exposed to different environmental contaminants (Seriani et al., 2012). In the present study, a significantly higher frequency of MN was observed in fish from Al-Huwaizah Marshes. Increased frequencies of MN were observed in fish populations exposed to abattoir effluent (Alimba et al., 2015), nutrients (Grisolia et al., 2009), heavy metals (Ayllon & Garcia-Vazquez 2000; Hussain et al., 2018) and pesticides (Bhatnagar et al. 2016). Despite Al-Sweap River receiving water from Al-Huwaizah Marshes, its inhabitant fish population exhibited a significantly low frequency of MN.

It is evident that although Al-Sweap River receives water from the Al-Huwaizah Marshes, the contamination effect on fish pollution from this river is not severe. Histopathological alterations have also shown a low pathological index at the Al-Sweap River for all organs. Marshes are considered to be important sinks for contaminants particularly metals, pesticides and nutrients (Reboreda & Caçador, 2007; Anjum et al., 2014), and this could be the explanation for the low pathological index in fish tissues and MN frequency in the blood of *C. carpio* from the Al-Sweap River.

5. Conclusion

The pollution effect was severe in the fish population from Al-Huwaizah Marshes, followed by Al-Hammar and Al-Sweap River, respectively. These findings have proven the reliability of histopathological alterations and MN induction as biomarkers of pollution in aquatic environments. This study further attests to the effectiveness of Al-Huwaizah Marsh and its biota in sinking and absorbing pollutants before the water enters the sea. Therefore, this study recommends contaminants analysis in the water, sediment and edible aquatic biotas such as fish in the Al-Huwaizah and Al-Hammar marshes to determine the safety upon their consumption.

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Conflict of interests

The authors have no conflict of interest to declare.

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