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Evaluation of the Impact of Polycyclic Aromatic Hydrocarbons on the Liver and Kidney of *Clarias gariepinus* Inhabiting Two Nile Delta Canals, Egypt

Noura Khattab[1*], Walaa T.S. Shalaby[1], Hassan M.M. Khalaf-Allah[2], Rashad E. M. Said[3]

[1]Department of Zoology & Entomology, Faculty of Science (girls), Al-Azhar University, Cairo, Egypt
[2]Department of Zoology, Faculty of Science (boys), Al-Azhar University, Cairo, Egypt
[3]Department of Zoology, Faculty of Science (boys), Al-Azhar University (Assiut branch), Assiut, Egypt

Corresponding author: nourakhattab90@gmail.com

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ABSTRACT

This research aimed to evaluate the effects of polycyclic aromatic hydrocarbons (PAHs) on the liver and kidneys of *Clarias gariepinus* as biomarkers. Specimens of *C. gariepinus* (18 individuals) were collected from two Delta Nile canals—Bahr Shebeen and El-Bahr El-Pharaounyin Al-Minufiya Governorate, Egypt, during July 2023. A total of 16 PAHs were assessed in the liver of the catfish, and histopathological changes in the liver and kidneys were examined. Results showed that the mean values of 16 PAHs—including naphthalene, acenaphthylene, acenaphthene, phenanthrene, anthracene, fluoranthene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, and indeno(1,2,3-cd)pyrene—were higher at site 2 than at site 1. In contrast, the mean values of fluorene and pyrene in the liver were higher at site 1 than at site 2. Additionally, the levels of benzo(a)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene were similar at both sites. Histopathological examination revealed that liver samples from site 1 exhibited congested blood vessels and signs of fatty degeneration, with hepatocytes showing patchy necrosis and bile stagnation. The liver of fish from site 2 showed fatty degeneration and hemorrhage of blood vessels. Microscopic observations of the kidneys from fish collected at site 1 indicated necrosis in renal cells, leading to degeneration of the renal corpuscles and vacuolation in the renal tubular cells. In contrast, the kidneys of fish from site 2 exhibited dilated renal tubules with vacuolar degeneration. This study determined that elevated levels of PAHs induced harmful histopathological alterations in the liver and kidneys of catfish. Therefore, technical treatments must be implemented for agricultural, industrial, and sewage waste discharged into the El-Bahr El-Pharaouny Canal.

INTRODUCTION

Aquatic pollution is a worldwide concern requiring immediate attention and management. It originates from various sources, including accidental chemical spills, industrial and sewage effluent discharges, agricultural runoff, domestic wastewater, and fuel from fishing boats **(Mendis *et al.*, 2015; Thanigaivel *et al.*, 2023)**. Therefore, water pollution constitutes a significant ecological and overall wellness issue in the Egyptian River Nile and its tributaries **(Mohamed *et al.*, 2013; Metwally *et al.*, 2023)**.

The Nile River, serving as Egypt's lifeline, is the nation's main source of fresh water, fulfilling all requirements for irrigation, and industrial use. The Nile receives numerous nonindicates and spot source emissions over its course through Egypt **(Elewa, 2010; Sabet *et al.*, 2017; Campbell, 2022)**. Currently, the alterations in Nile water and its distributaries are mainly attributed to a mix of these contaminants, with human activities significantly impacting water quality **(Mapfumo *et al.*, 2002; Abdelsalam *et al.*, 2024)**. Some of these impacts stem from polluting activities, including the release of municipal, manufacturing, urbanized, and additional effluent into the watercourse **(Fernandez *et al.*, 2002; Tariq & Mushtaq, 2023)**.

According to **GAFRD (2021)**, the natural fishery resources in Egypt are diminished due to aquatic pollution **(Barakat *et al.*, 2012; El-Maksoud, 2022)**. Fish are therefore an important and promising species for the biomonitoring of water pollution **(Okwuosa *et al.*, 2019; Iyiola *et al.*, 2024)**.

Polycyclic aromatic hydrocarbons (PAHs) are significant environmental contaminants which are classified as high-risk pollutants by the USEPA due to their omnipresence and toxicity. They adversely affect ecosystems and carcinogenic, teratogenic, and mutagenic properties **(Honda & Suzuki, 2020; Marvin *et al.*, 2020; da Silva Junior *et al.*, 2021; Mallah *et al.*, 2022; Bai *et al.*, 2024)**.

PAHs are a category of endocrine-disrupting chemicals (EDC), characterized by lipophilic properties, low aqueous solubility, and greater solubility in fat **(Jurewicz *et al.*, 2013; Liu *et al.*, 2020)**. PAHs are released into the environment via insufficient combustion such as fossil fuels, coal, wood, oil/gas, solid wastes, petrol and diesel, tobacco, vaporization of synthetic chemicals, cocking, and vehicle exhausts fumes **(Jurewicz *et al.*, 2013; Liu *et al.*, 2020)**. Consequently, PAHs easily dissolve, facilitating their transport and uptake by aquatic organisms **(Mojiri *et al.*, 2019; Suresh *et al.*, 2024)**. This is particularly significant given the sensitivity of *C. gariepinus* to the presence of xenobiotics **(Rand *et al.*, 2020)**.

The presence of *C. gariepinus* at the bottom of freshwater ecosystems indicates its tolerance to a wide range of pollutants, making it a valuable monitor species for tracking pollution. Therefore, the current research aimed to assess the influence of PAHs on the liver and kidneys of *C. gariepinus* as biomarkers in this sentinel freshwater species.

MATERIALS AND METHODS

1. *C. gariepinus* collection

Specimens of *C. gariepinus* (18 specimens) were retrieved during July 2023 from different localities of two Delta Nile canals; 9 specimens (5♀ 4♂) from Bahr Shebeen Canal (33 – 81cm in total length and 1 -3.5kg in weight) and 9 specimens (6♀+ 3♂) from El-Bahr El–Pharaouny Canal (36 – 68cm in total length and 1 -2.5kg in weight) formed the materials for the present study. The main fishing methods for collecting catfish were trammel nets and basket traps (Gwabi). Fish were examined fresh and transported to the Laboratory of Zoology and Entomology, Department of Zoology & Entomology, Faculty of Science (Girls' Branch), Al-Azhar University, Nasr City, Cairo, Egypt for further studies. Fish were classified in the laboratory according to the method of **Bishai and Khalil (1997)**.

Fresh samples were rinsed in distilled water to eliminate any external contaminants. Dissection was carried out on these samples using robust and sharp instruments due to the full ossification of the catfish. The liver was removed from fish samples (20g) wrapped in aluminum foil and stored in a deep freezer until examination. The samples were subsequently blended and kept in airtight containers before the extraction process for PAHs analyses.

2. Determination of 16 PAHs in liver of *C. gariepinus*

To quantify the 16 PAHs in the liver of *C. gariepinus*, an analytical and separation approach using high-performance liquid chromatography (HPLC) was conducted with an Agilent 1260 series system. Separation was performed on a Zorbax Eclipse PAHs column (4.6mm x 150mm, 5µm). The mobile phase consisted of water (A) and acetonitrile (B) at a flow rate of 2.0ml/ min, with the diode array detector calibrated at 220nm. An injection volume of 5µl was used, and the column temperature was maintained at 25°C. PAH standards from EPA 610 were obtained from Supelco (Bellefonte, PA, USA) at the Laboratory of the National Research Center.

Two grams of liver samples were placed in a clean extraction vessel (50ml flask), to which 20ml of acetone was inserted. The flask was subjected to sonication for 30 minutes. Purification was conducted using solid phase extraction (SPE) with C18 mini-column cartridges (Clifton TM, SW3H, UK). The mixtures were vigorously agitated and then allowed to decant for half an hour **(Sarrazin *et al.*, 2006)**. The extracts were subsequently transferred to HPLC for fingerprint analysis using a diode-array detector (DAD) and a fluorescence detector (FLD).

3. Statistical analysis

The data obtained from the HPLC analytical and separation technique system at the two studied sites were presented as mean ± standard deviation and statistically analyzed using a Student's T-test (Levene's test) via the Statistical Package for Social Sciences (SPSS) (IBM SPSS Statistics Version 22; SPSS Inc., IL, USA) to compare the means.

4. Histopathological investigation

For histopathological studies, anesthetized specimens were dissected; the liver and kidney were excised and inspected. Organs were sectioned into 5mm thick sections and promptly fixed in alcoholic Bouin`s fluid for a minimum of 48 hours, thereafter dehydrated in rising concentrations of ethyl alcohol, cleaned in xylene, and embedded in paraplast wax (M.P.: 58°C). Transverse slices were prepared at the thickness of 4-6µm, organized, and stained with haematoxylin and eosin (H&E) stain according to routine histological technique **(Suvarna *et al.*, 2012)**. The stained slides were examined under the light microscope (XSZ-N107T) at various magnifications, thereafter photographed with a digital camera (Toup Cam, Ver. 3.7) and described.

RESULTS

1. PAHs in the liver of *C. gariepinus*

The results shown in Table (1) indicate that the mean values of Nap, Acpy, Acp, Phen, Anthr, Fl, Baa, Chry, Bbf, Bkf and Ip in the liver of *C. gariepinus* were greater at site 2 compared to site 1. The mean values of fluorene and pyrene in the liver were higher at site 1 than at site 2 (0.648± 0.578 and 58.660± 10.526). Fluoranthene, benzo(a)Pyrene, dibenzo(a,h)anthracene, and benzo(g,h, i)Perylene were comparable in liver of *C. gariepinus* from the two locations.

T-test (Table 1) revealed that the mean concentration of all PAHs in the liver exhibited no significant variations between the two examined canals ($P>0.05$) with the exception of benzo(b)fluoranthene, which exhibited statistically significant differences between the two investigated canals ($P<0.05$).

Table 1. Mean ± SD of PAHs in the liver (mg/g) of *C. gariepinus*, collected from the two investigated sites

PAHs in liver (mg/g)	Site 1	Site 2	Sig.
Naphthalene	0.363±0.160	0.520±0.288	NS
Acenaphthylene	0.0±0.0	0.532±0.615	NS
Acenaphthene	0.122±0.211	0.685±0.741	NS
Fluorene	0.882±1.527	0.648±0.578	NS
Phenanthrene	0.669±0.192	1.340±1.106	NS
Anthracene	8.353±4.565	11.431±8.488	NS
Fluoranthene	1.447±0.905	1.497±1.298	NS
Pyrene	129.049±56.319	58.660±10.526	NS
Benz(a)anthracene	5.0158±2.601	6.234±1.702	NS
Chrysene	3.4250±2.724	9.351±10.365	NS
Benzo(b)fluoranthene	31.038±18.056	90.822±31.236	*
Benzo(k)fluoranthene	2.783±2.673	8.993±6.298	NS
Benzo(a)Pyrene	7.741±5.448	7.206±5.707	NS
Dibenzo(a,h)anthracene	2.852±1.385	2.047±0.512	NS
Benzo(g,h,i)Perylene	2.122±2.952	2.093±2.108	NS
Indo(1,2,3-cd) Pyrene	2.225±3.854	4.183±4.644	NS

*: The mean difference is significant at the 0.05 levels
NS: The mean difference is not significant.

2. Histopathology of liver

Examination of hebetic sections from *C. gariepinus* at site 1 revealed well-structured hepatocytes aggregate in clusters, interspersed by blood sinusoids, and organized into anastomosing laminae and rings encircling a central vein. Each hepatocyte exhibited polygonal morphology and possesses a substantial, spherical nucleus featuring conspicuous nucleolus. The blood sinusoids are bordered with a layer of flattened epithelial cells (endothelial cells) featuring elongated nuclei. Branches of hepatic portal vein and bile duct are observed in the liver. The liver compartments are delineated by minimal connective tissues (Fig. 1A).

Nevertheless, subtle histological changes in the hepatic tissue's architectural pattern (Fig. 1) and congested blood vessels were observed (Fig. 1A). The liver showed indications of fatty degeneration, where fine vacuoles appeared in the cytoplasm of hepatocytes (Fig. 1A, B). Hepatocytes exhibited patchy necrosis, which led to disorganization of liver tissue (Fig. 1B, C). In addition, bile stagnation was also found (Fig. 1C).

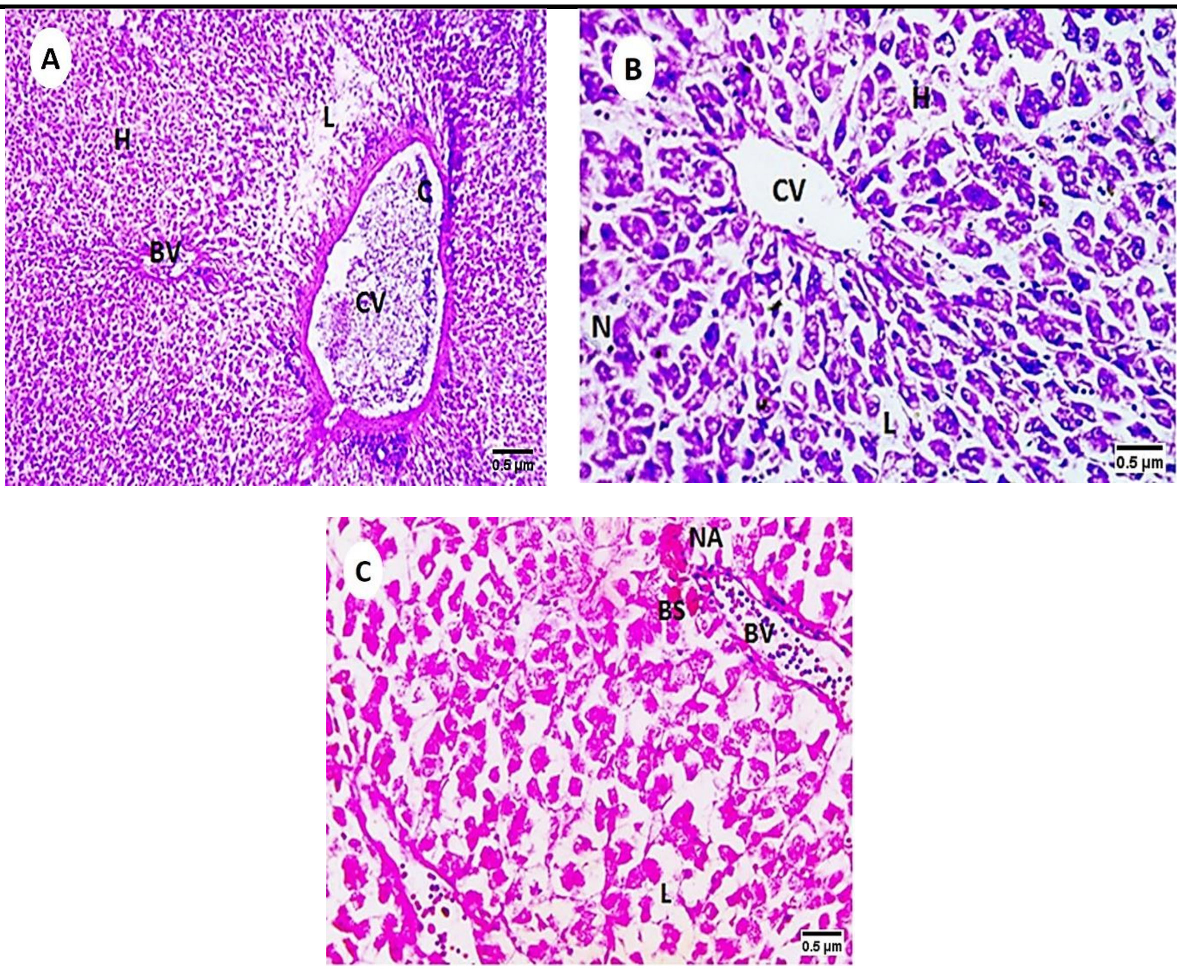

Fig. 1. Photomicrograph of liver section from *C. gariepinus* obtained from site 1 showing: **A.** Hepatocytes that aggregate in masses (H), around a central vein (CV) and blood vessels (BV) and showing little abnormal liver structure; liposes (L) and congested blood vessel (C). (H&E 100x); **B.** Hepatocytes that aggregate in masses (H), around a central vein (CV) and show little abnormal liver structure; liposes (L) and necrosis (N). (H&E 400x). **C.** minor alterations in liver structure; bile stagnation (BS) and necrotic area (NA). (H&E 400x)

Conversely, the liver of fish at the second site revealed remarkable histopathological alterations (Fig. 2). The severely dilated and congested blood vessels showed signs of severe lymphocytic infiltration, which caused hemolysis and melano-macrophage aggregation. This congestion was accompanied by adhesive blood cells and hemolysis with blood vessels (Fig. 2A). The liver showed evidence of fatty degeneration, characterized by the presence of fine vacuoles inside the cytoplasm of hepatocytes (Fig. 2B). Additionally, the hemorrhage of blood vessels was also observed (Fig. 2B). Tissue fibrosis was also prevalent intermixed with a large number of leukocytes (Fig. 2C).

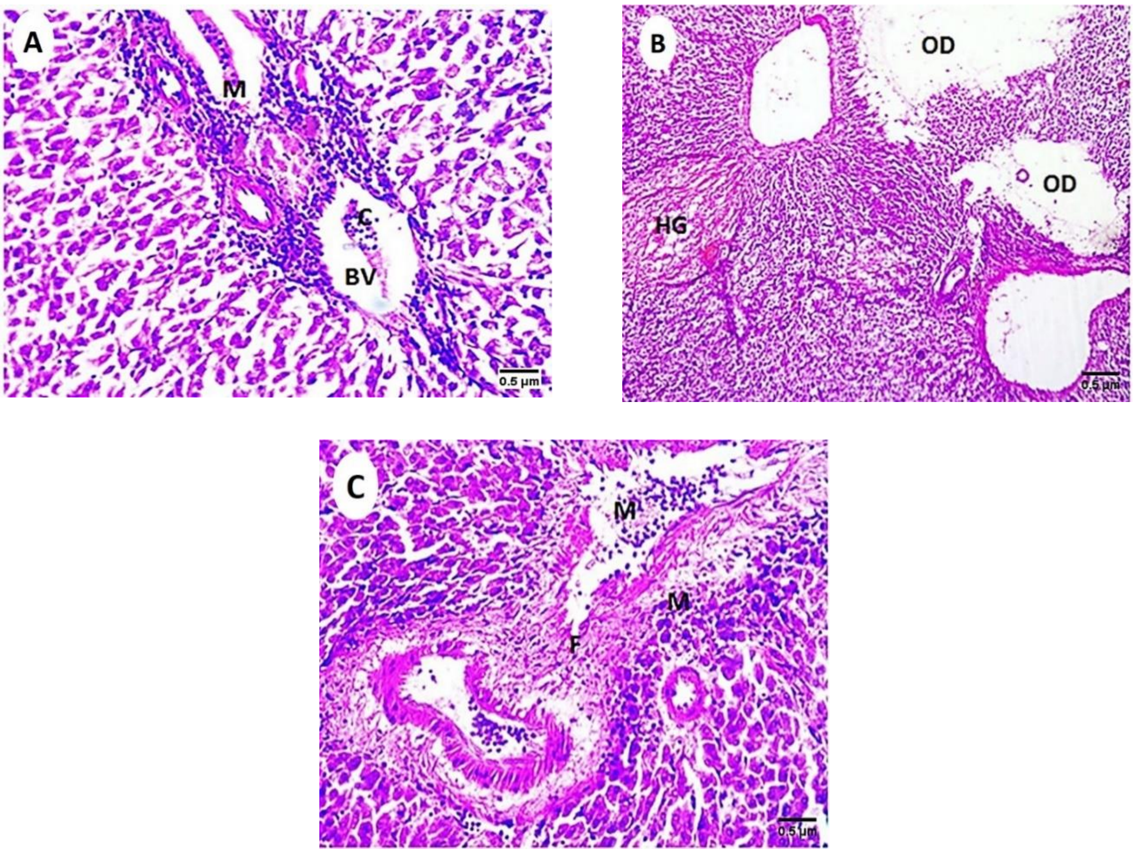

Fig. 2. Photomicrograph of liver section from *C. gariepinus* collected from site 2 showing: **A.** More abnormal liver structure; congested blood vessel (C) leading to hemolysis and aggregation of melano-macrophages. (H&E 400x); **B.** More deteriorated liver structures; hepatocyte degeneration leads to a significant accumulation of fat that produces massive oil droplets (OD) beside the hemorrhage (HG) of blood vessels. (H&E 100x); **C.** Detrimental effects in liver structure; hemolysis and aggregation of melano-macrophages (M) with fibrosis (F). (H&E 400x)

3. Histopathology of kidney

Histological analysis of the kidney of *C. gariepinus* obtained from site 1 demonstrated that the predominant histological features of the kidney consist primarily of renal tubules and renal corpuscles. The renal tubular comprised of simple columnar epithelial cells, while, the renal corpuscle has glomerulus within Bowman's capsule. Bowman's capsule is composed of bilayer epithelium and features a crescent-shaped lumen referred to as the capsular space. The renal tissues are richly vascularized and contain hematopoietic tissue. The renal tubules consisted of proximal tubules, distal tubules, and collecting ducts. The proximal tubules lined with tall columnar epithelial cells featuring basal nuclei, whereas distal tubules were covered by big, relatively transparent columnar epithelial cells with central nuclei. The collecting duct exhibited a greater diameter than the distal segment, comprising columnar epithelial cells with basal nuclei (Fig. 3A).

The microscopic observation in the kidney of fish obtained from low levels of PAHs at site 1 showed some histopathological alterations in the renal tissue; necrosis in renal cells leading to a degeneration of renal corpuscle to form vacuolation in renal tubular cells (Fig. 3B). Severe degeneration in the renal tubules and aggregation of melano-macrophages with hemosiderin granules, glomerular atrophy, and necrotic areas were also detected (Fig. 3C).

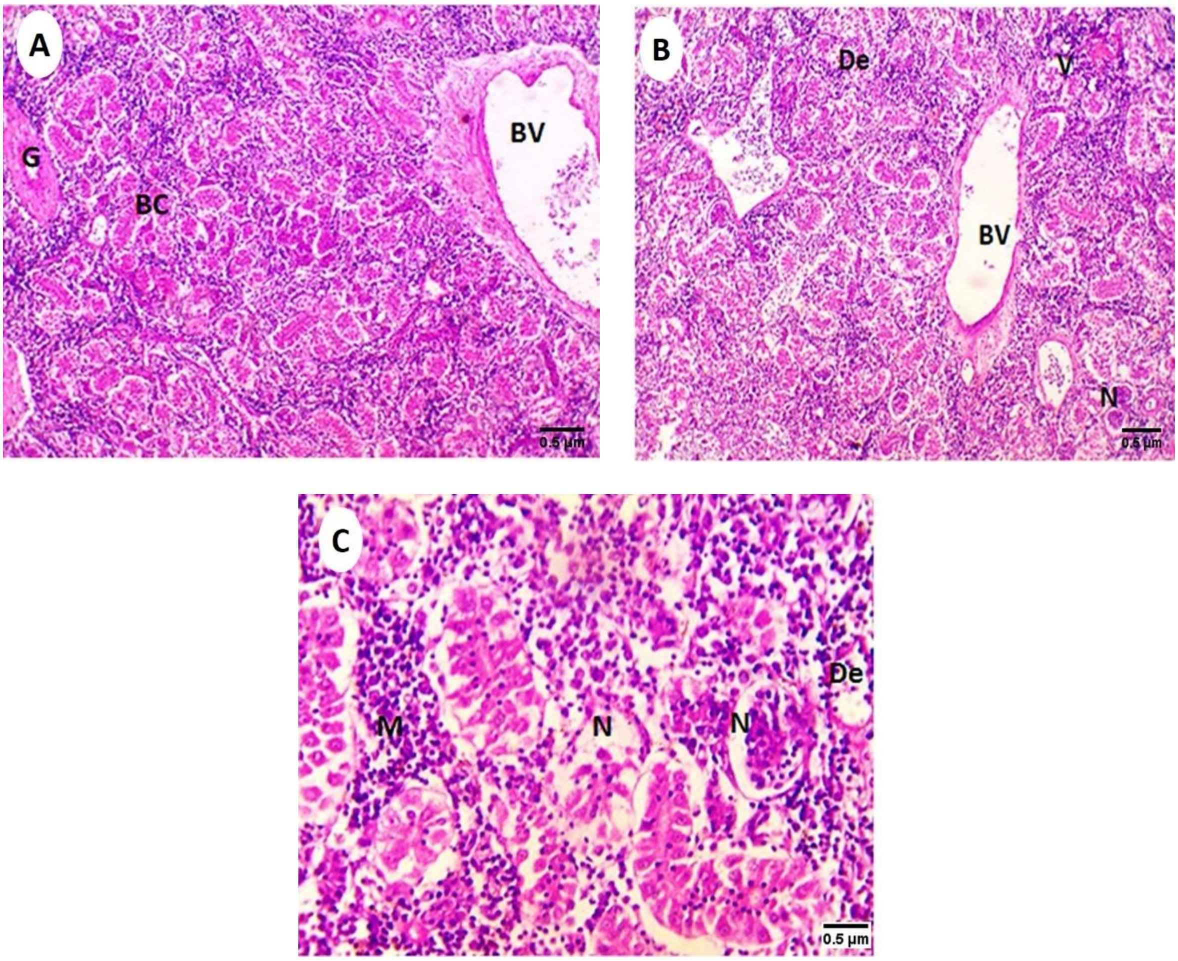

Fig. 3. Photomicrograph of T.S, within the renal structure of *C. gariepinus* obtained from site 1 showing: **A.** The renal corpuscle is consisting of a glomerulus (G) encased in Bowman's capsule (BC) and the renal tissues also have numerous blood vessels (BV). **(**H&E 100x); **B.** Little abnormal kidney structure: necrosis (N) in renal cells leading to degeneration (De) of renal corpuscle to form vacuoles (V). (H&E 100x); **C.** Mild abnormal kidney structure: necrosis (N) in renal cells leading to degeneration (De) of a renal corpuscle and aggregation of melano-macrophages (M). **(**H&E 400x)

On the other hand, the kidney of fish collected from high levels of PAHs at the second site showed many renal tubules were dilated and renal tubular epithelium showed vacuolar degeneration, leading to tubular necrosis. In addition, glomerular changes were noticed such as glomerular shrinkage and fibrocytes were detected surrounding the Bowman's capsule leading to peri-glomerular fibrosis, as well as necrosis of endothelial cells and renal hemopoietic tissue, indicating severe renal damage was also observed (Fig. 4A,B). Severe congestion, blood hemolysis, aggregation of melano-macrophages with hemosiderin granules deposits and separation or detachment of epithelial cells from the renal tubules basement membrane resulted in edema and atrophy of both renal tubules and glomeruli were noticed (Fig. 4C).

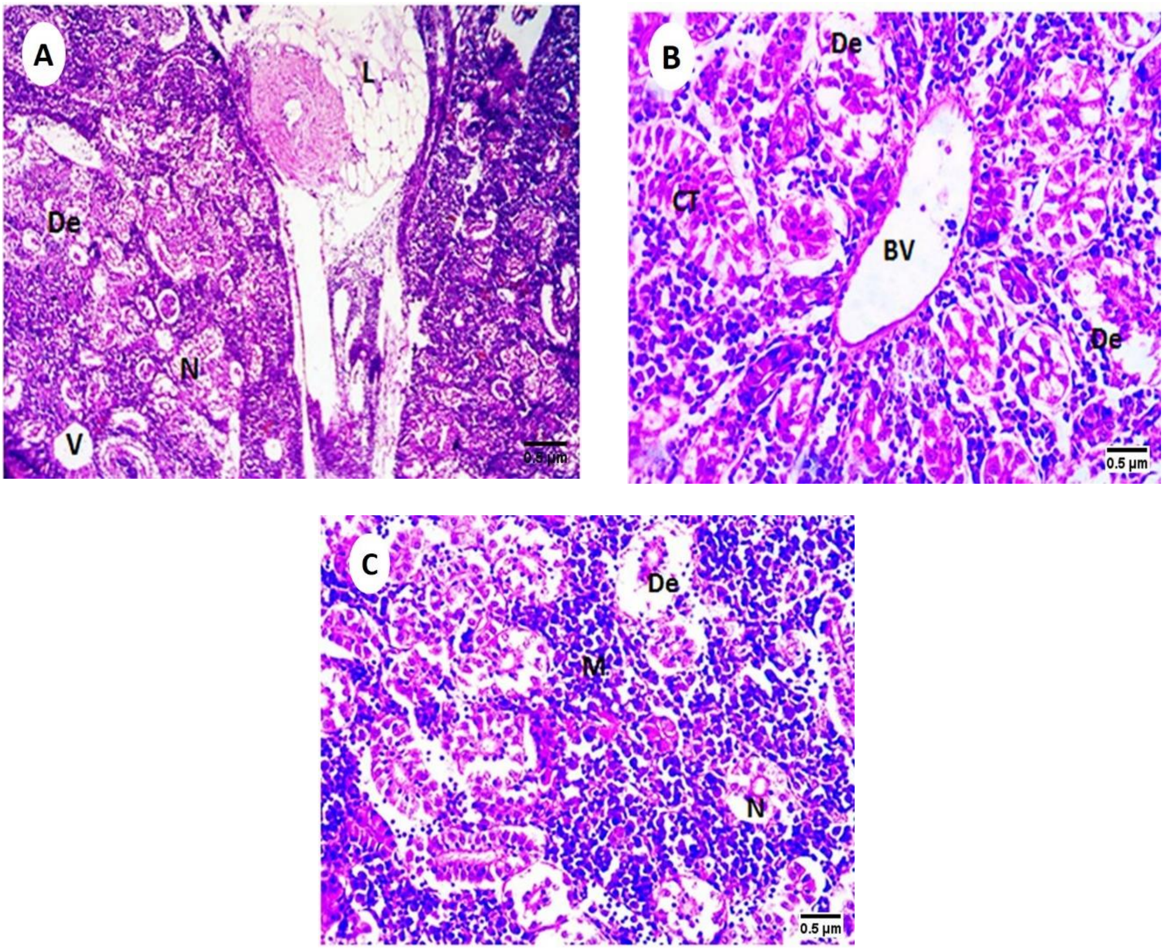

Fig. 4. Photomicrograph of T.S, within the kidney of *C. gariepinus* collected from site 2 showing: **A.** more abnormal kidney structure: necrosis (N) in renal tubular cells leading to degeneration (De) of renal tubules to form vacuoles (V) or liposes (L). (H&E 400x); **B.** More abnormal kidney structure: degeneration (De) of collecting tubule (CT) and liposes of blood vessels (BV). (H&E 400x); **C.** More abnormal kidney structure: necrosis (N) in renal tubular cells leading to degeneration (De) and aggregation of melano-macrophages (M). (H&E 400x)

DISCUSSION

In the current investigation, *C. gariepinus* was selected to assess the accumulation patterns of PAHs in the liver. Therefore, the present work demonstrates that the liver has the potential to accumulate all 16 PAHs analyzed, as it is the body's primary detoxification organ and has higher metabolic activity than other organs. PAHs are lipophilic (fat-soluble) compounds, and due to the liver’s high lipid content, it tends to accumulate these substances more than other tissues by metabolic activity process **(Ayandiran *et al.,* 2022)**. **Rimayi and Chimuka (2019)** detected that the liver of *C. gariepinus* contains enzymes capable of converting PAHs into more water-soluble metabolites through biotransformation. However, some of these metabolites may still be toxic and can accumulate in the liver. This interpretation implies that exposure to contamination results in liver damage, manifesting as elevated liver weight and fat accumulation, ultimately leading to cell necrosis **(Hinton *et al.,* 2017; Topić Popović *et al.,* 2023)**. Regarding the specific composition of PAHs in liver tissue, the samples were gripped by four aromatic benzene-ring PAHs in the two investigated sites with the highest overall concentration of benzo (b)fluoranthene observed at site 2 (252mg/ g). Benzo(b)fluoranthene consists of a benzene ring fused with an acephenanthrylene ring, a low soluble form, easily adsorbed to organic matter, indicating that PAHs are of pyrolytic (combustion) origin **(NCBI, 2024)**. The main cause of the benzo (b)fluoranthene at site 2 is probably runoff from nearby agricultural land and untreated wastewater spilled into the canal. Therefore, the concentrations of PAHs obtained in the liver of *C. gariepinus* were high; this is due to the lipophilic nature of PAHs, which allows them to reside and accumulate in fatty tissues. According to **Nasr *et al.* (2010)**, they enter fish via ingestion, cutaneous absorption and respiration, either directly from water and sediments (waterborne exposure) or via contaminated food in the food chain (dietary exposure). These findings align with those of **Yancheva *et al.* (2016)**.

The current findings surpass the results documented by **Nasr *et al.* (2010)**. The total concentrations of PAHs in fish samples from the same location ranged from 37.168 mg/g in El-Sarsawia Canal to 201.925 mg/g at site 1, showing differences between sites. The current results indicate that the Σ16 PAHs at site 1 were recorded at 594.97 mg/g, with a maximum total concentration level of Σ16 PAHs reaching 618.743 mg/g in the liver samples from site 1. The fish primarily contained high molecular weight PAHs (4-ringed). Research by **Baumard *et al.* (1998)** demonstrated that fish have a strong tendency to bioaccumulate 4-, 5-, and 6-ringed PAHs compared to 2- and 3-ringed PAHs. This is attributable to the higher octanol-water partition coefficient (Kow) and the increased solubility of lower molecular weight PAHs **(Porte & Albaiges, 1993)**. The accumulative content of pyrene in the liver of *C. gariepinus* from site 1 recorded abundant anomalous hydrocarbon than pyrene in the liver tissue of *C. gariepinus* at site 2 and other PAHs. This trend could be since other PAHs are more soluble and degradable **(Helfinalis *et al.,* 2021)**. However, the high molecular weight (202.3) and less volatilization of pyrene originate from burning the ash in agricultural lands surrounding site 2. Furthermore, pyrene may have emerged from the condensation of low aromatic rings of PAHs at elevated temperatures **(Na *et al.,* 2021)**. In accordance, pyrene was found to be the highest abundant hydrocarbon in the study of **Soliman *et al.* (2023)**, suggesting the petroleum contamination by existing oil refineries in the Suez Bay, Egypt.

Ammar *et al.* (2017) detected pyrethroid and carbamate pesticide residues in *Oreochromis niloticus* and water samples collected from four regions of site 2. The mean concentrations of pesticides of residue levels of identified pyrethroid and carbamate pesticides found in fish tissues and their maximum residue limits (MRLs) presented in the muscles of fish were greater than those observed in water samples. Cypermethrin was detected in muscles of fish samples from Kafr El-Khadra (0.5mg), Kafr-Fesha (0.3mg), and Hozet-Menouf (0.3mg) sites, represented as site 2 in this study; cypermethrin was detected in only one sample (25%) from Kafr-Fesha and Hozet-Menouf. Lambda-cyhalothrin was also detected in fish from Kafr-Fesha (0.5mg), Hozet-Menouf (0.65mg), and Shanshour (0.4mg). It was detected in only one sample (25%) from Kafr-Fesha and Shanshour sites.

The finding indicated that the mean values of all PAHs in the liver exhibited no significant differences except benzo(b)fluoranthene that gave statistically significant differences between the two investigated canals, due to PAHs that accumulated gradually in the liver than in the habitat over time. Additionally, the ability of catfish to accumulate pollutants without suffering significant harm or mortality is one of the criteria for selecting an appropriate bioindicator (**Triana *et al.*, 2023**). This suggests that while most PAHs were similarly distributed between the two canals, benzo(b)fluoranthene had a distinct pattern of accumulation, possibly due to differences in local sources, environmental conditions, or biological factors affecting its distribution and metabolism

Histopathological changes can act as markers for assessing the impact of different xenobiotic contaminants on aquatic organisms and can thus reflect the general health of the ecosystem's population **(Ibrahim & Omar, 2013; Yancheva *et al.*, 2016; Oladunjoye *et al.*, 2021)**. Extensive researches have been conducted on histopathological changes in fish and shellfish, and these changes have been proposed as biomarkers for environmental monitoring. The liver functions as the principal organ for detoxification **(Louiz *et al.*, 2018)**. Accordingly, histological changes in the liver can serve as indicators of previous exposure to certain environmental stressors **(Annabi *et al.*, 2018)**.

The liver, the largest gland in fish, executes numerous intricate activates. This encompasses the excretion of waste products, secretion of bile, synthesis of proteins including fibrinogen, globulins, albumin, and clotting factors, as well as the storage of lipids, vitamins A & B and glycogen. Additionally, the liver is involved in phagocytosis of foreign particles, detoxifies lipid-soluble substances and drugs, conjugates toxic substances and steroid hormones, esterifies free fatty acids into triglycerides and metabolizes proteins, carbohydrates, lipids, hemoglobin and drugs. It also contributes to hemopoiesis during embryonic development and potentially in adult fish **(Dellmann & Eurell, 1998)**. IN addition, the liver is essential for the vitellogenesis of oocytes and energy production during spawning **(Toru & Shozo, 1998)**.

This study revealed that the liver of *C. gariepinus* from site 1 exhibited congested blood vessels and fatty degeneration, resulting in the formation of large vacuoles or oil droplets. The vacuoles in the hepatocyte cytoplasm contain lipids and glycogen, which are associated with the liver's stander metabolic process **(Haque *et al.*, 2017)**.

Abiona *et al.* (2019) asserted that exposure to pollutants results in fatty degeneration and vacuolization in hepatocytes. While, **Pacheco and Santos (2002)** identified increased vacuolization in hepatocytes as indicative of a degenerative process, likely due to exposure to contaminated water. This finding aligns with the results reported by **Getnet *et al.* (2024)**. Moreover, vacuole formation was considered by **Hadi and Alwan (2012)** as a cellular defense mechanism toward toxic compound to hepatocytes, responsible for sequestering detrimental material and preventing disrupting of the basic functioning of them cells.

The liver of fish from site 2 exhibited signs of fatty degeneration, where fine vacuoles appeared in the cytoplasm of hepatocytes. The degeneration of hepatocytes gave a high accumulation of fats which made the hepatocytes fatty metamorphosed forming large vacuoles or oil droplets. **Sayed *et al.* (2023)** and **Hamed *et al.* (2024)** similarly demonstrated that pyrogallol exposure resulted in hebetic injury in *C. gariepinus*, leading to cellular changes such as hepatocyte hydropic degeneration, melano-macrophage formation, vacuolated hepatocytes, engorged blood vessels, pronounced structural deformation, and hemorrhage.

This study demonstrated degeneration and necrosis in the hepatic cells of *C. gariepinus*. Numerous investigations have illustrated vacuolar degeneration and necrosis in liver cells. According to **Khattab *et al.* (2002)** and **Haque *et al.* (2017)**, vacuolar degeneration may result from the direct impact of toxic substances on cell membranes. **Fouda and Azab (2003)** characterized neoplasia as the consequence of fibrocytes and leukocytes encapsulating necrotic area.

Pacheco and Santos (2002) proposed that metabolic impairment and biochemical degenerative processes can be recognized by lesions in hepatic tissues, especially through changes in hepatocytes. **Fouda and Azab (2003)** emphasized the liver's crucial role in the impact of pollutants on fish, highlighting it as the primary organ for the biotransformation of organic xenobiotics and the elimination of deleterious trace materials.

Padmini and Usha Rani (2009) noted that lipids might signify a disruption in lipid metabolism or a partial alteration in their morphologies. The hepatocytes of fish residing in the contaminated Encore estuary exhibited oxidative stress attributable to reactive oxygen species (ROS) induced by heavy metals pollution **(Rajeshkumar *et al.*, 2014)**.

The kidney is essential for homeostasis by eliminating waste from the blood and facilitating reabsorption, which helps regulate blood and body fluid volume and pH, as well as erythropoiesis **(Iqbal *et al.*, 2004)**. In teleost fish, the kidney is among the initial organs impacted by waterborne pollutants **(Peebua *et al.*, 2006)**.

The current study revealed that kidney of *C. gariepinus* from site 1 showed necrosis in renal cells leading to degeneration of renal corpuscle to form vacuolation in renal tubular cells. Severe degeneration in the renal tubules was noticed and aggregation of melano-macrophages with hemosiderin granules. According to the present results, the degenerative process results in tissue necrosis. Necrosis of renal tubular cells markedly affects metabolic functions and aggravates metabolic disorder in fish **(Naeemi *et al.*, 2013; Avijit *et al.*, 2021)**. The current findings are consistent with those observed in *C. batrachus* **(Chandra *et al.*, 2015)** and *C. gariepinus* **(Hamed *et al.*, 2024)**.

This study found that tubular degeneration and necrosis were observed in the kidneys of *Clarias gariepinus* from site 2, indicating damage due to PAHs exposure. Similar findings were reported by **Abubakar *et al.* (2019)**. The increased PAHs concentration led to glomerular shrinkage and hemorrhage, likely resulting from cellular degeneration and buildup of edematous fluid in the interstitial matrix **(Hadi & Alwan, 2012)**.

Kavitha *et al.* (2023) reported that hemorrhages and congestion in kidneys exposed to polluted water impair cell membrane permeability and inhibit ion-transporting systems. This disruption affects fluid transport into and out of cells. According to **Hadi and Alwachi (1995)**, this process integrates with cell membranes, leading to significant fluid filtrate and the dispersion of serum albumin and red blood cells into the interstitial matrix due to break down of capillary endothelium**.**

CONCLUSION

The current study found that the catfish are exposed to different levels of hydrocarbons in their natural environment. Histopathological evidence revealed varied and numerous histopathological deteriorations, both in the liver and kidney of *C. gariepinus*, due to water-borne PAHs exposure. These findings implied the efficiency of these tissues as key organs in ecotoxicological studies. Consequently, agricultural, industrial, and sewage pollution that is dumped into freshwater bodies must be treated technically to protect aquatic animals and natural resources.

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