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Impact of Polycyclic Aromatic Hydrocarbons on the Ovary of *Clarias gariepinus* in Two Canals of Al-Minufiya Governorate, Egypt

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ABSTRACT

The study aimed to quantify polycyclic aromatic hydrocarbons (PAHs) in the sediments and to assess their impact on the ovary of the catfish (*Clarias gariepinus*) obtained from two Delta Nile Canals, Bahr Shebeen (Site 1), and El-Bahr El-Pharaouny (Site 2) Canals in Al-Minufiya Governorate, Egypt. Eight female catfish specimens were collected from various localities at two examined sites during July 2023. Furthermore, Σ16 PAHs were quantified in sediments. Some female sex hormones were evaluated. Morphology, maturation and histopathological changes in the ovary were elucidated. The results indicate that the mean values of PAHs including, naphthalene, phenanthrene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)Pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)Perylene were higher in sediment from site 2 compared to site 1. In contrast, the mean values of pyrene, benz(a)anthracene, and indo(1,2,3-cd) pyrene were higher in sediment from site 1 than site 2. Fluorene value (1.006±1.743) was exclusively detected in sediment at site 2. Acenaphthylene, acenaphthene, anthracene and fluoranthene were completely absent in the sediments of the two investigated sites. The mean value of progesterone (ng/ml) was higher in serum of fish collected from site 1 in comparison with site 2. On the other hand, the estradiol (E_2) (pg/ml) was greater in serum of fish obtained at site 2 than site 1. The ovarian morphology of fishes from site 1 exhibited a normal structure, while slight abnormalities were observed at site 2. The ovary of the catfish from site 1 exhibited the lowest incidence of atretic oocytes, whereas site 2 displayed the lowest percentage of mature oocytes alongside the highest occurrence of atresia. Microscopic examinations revealed degenerative alterations (atresia) in certain oocytes leading to the adherence of their cellular covering. This study revealed that elevated levels of PAHs result in heightened histopathological changes in the ovaries of the catfish, therefore serving as effective biomarkers for assessing PAHs in freshwater ecosystems.

INTRODUCTION

The Nile River is recognized as the longest river in the world and is considered one of the most significant rivers globally. It serves as a vital lifeline for Egypt. Throughout recorded Egyptian history, the Nile has had a profound influence on the economy, culture, public health, social life, and politics **(Abdalaal *et al.*, 2024)**. The development of canals and drains throughout the Nile River valley, encompassing its Delta, had encouraged farmers to establish villages along the banks of the canals to the diminished extent of the drains. Both

canals and drains have been contaminated by effluent from these growing communities. This has transpired when the canal or drain water is utilized for washing dishes, cleaning animals, discharging urine and/or human feces and disposing of garbage and deceased animals. Certain canals and drains have been polluted with heavy metals, herbicides, insecticides, fertilizers, potential residential and industrial waste, and other chemicals such as PAHs **(Nasr *et al.*, 2010; Zaki *et al.*, 2014; Azab *et al.*, 2019)**.

Bahr Shebeen Canal (Site 1) is a significant aquatic and fishing resource across Al-Menoufia Governorate in Egypt. It is a semi-autonomous aquatic habitat linked to the Nile via Alrayah Elmenoufi near the Barrage, characterized by its modest depth (2-3m) and small width (30m) as an irrigation canal. It extends over 80km through the Egyptian Delta, flanked by two major cities, various villages, and arable lands, while riparian plant is scarce due to human interference, particularly near urban areas owing to shoreline protection measures **(Khallaf *et al.*, 2018, 2023)**.

El-Bahr El-Pharaouny canal (Site 2) in Al-Menoufia Governorate is a closed water body, measuring roughly 32km in length, with a width ranging from 100 to 300m; a depth of 3 to 7 meters; and a total area of over 2500 feddans. It holds historical and economic significance in the realms of fishing and agriculture. It was linked to the Nile River, but has since been obstructed due to the filling of certain sections for road construction or agricultural use. It received untreated domestic sewage from several towns and villages, together with agricultural waste (salts, fertilizers, and pesticides), which significantly affects ambient water quality and lack of fisheries **(GAFRD, 2011; Gohar *et al.*, 2015)**.

The pollution levels are escalating daily due to the absence of an adequate drainage system for industrial facilities and residential communities situated along its banks. The escalating pollution levels are significantly endangering human health and rendering the water detrimental for irrigation and fishery purposes **(Ghannam *et al.*, 2014; Sabae *et al.*, 2014)**. PAHs are organic compounds characterized by the presence of two or more fused benzene rings, originating from both natural and manmade sources. They are pervasive environmental pollutants that exhibit deleterious biological effects, encompassing toxicity, mutagenicity, and carcinogenicity, attributable to their widespread occurrence, recalcitrance to degradation, potential for bioaccumulation, and carcinogenic properties **(Anyahara, 2021; Ejiako *et al.*, 2022; Emoyoma *et al.*, 2024)**.

Sediments are considered a significant medium for aquatic monitoring. Alongside water, they facilitate the transport of nutrients and pollutants within aquatic environments and habitats essential for biological processes **(Dai *et al.*, 2022; Yin *et al.*, 2024)**. Consequently, sediments establish a crucial connection between chemical and biological processes and were known to sequester hydrophobic chemical pollutants entering water bodies **(McCready *et al.*, 2006)**. Since sediment tends to accumulate PAHs and other pollutants, especially organic matter, this accumulation may endanger aquatic organisms, even at minimal doses **(Ghandourah, 2022)**. Therefore, fish occupy elevated positions in the food web and can amass significant quantities of various chemical pollutants from water, sediment, and their diet, resulting in contaminant concentrations that may exceed those detected in water, sediment, and food **(Osman *et al.*, 2007; Tierney *et al.*, 2013)**.

Reproduction, considered the paramount function in aquatic organisms impacted by persistent toxicant exposure, via persistent organic pollutants (POPs) (**Satkar *et al.*, 2024)**. Over the past decade, the escalating dispersion of pollutants in aquatic ecosystems has been observed to exert cumulative effects on fish organs, ultimately impairing organ functions such as reproduction, jeopardizing growth and survival and diminishing species diversity across various global regions **(Yacoub *et al.*, 2021)**. The ovaries of female fish that do not spawn become atretic shortly after the spawning period **(Solé *et al.*, 2016)**.

Steroid sex hormones are crucial for the sexual differentiation and reproductive physiology of fish. Consequently, it was unsurprising that chemical pollutants exhibiting steroid-like activities have been implicated in atypical manifestation of gonadal intersex disorders and other gonadal anomalies in fish **(Scholz & Klüver, 2009)**.

Hinton *et al.* (2018) asserted that histological changes in certain target organs are extensively utilized as sensitive indicators for xenobiotic impacts. Moreover, various biomonitoring programs have employed histological alterations in diverse fish organs as biomarkers in assessing the ecological health of aquatic environments **(Pinto *et al.*, 2009; Yancheva *et al.*, 2016; El-Ghazaly *et al.*, 2017)**. The patterns of pollutant accumulation in fish and other aquatic species are influenced by both uptake and elimination rates **(Savoca & Pace, 2021)**. A large body of research has explored the effects of water contamination on fish reproduction, confirming detrimental effects on the reproductive capacity of fish organs **(Getnet *et al.*, 2024)**. Various contaminants, including heavy metals, pesticides, and diverse bacterial strains, exert histopathological effects on the reproductive tissues of fish **(Elgaml *et al.*, 2019)**. However, exposure to persistent organic pollutants (POP) via PAHs can result in acute or chronic toxicity to fish organs, particularly the reproductive organs, leading to reproductive failure **(Johnson *et al.*, 2013)**.

PAHs are dissolved in and transported by water, and are rapidly absorbed by aquatic creatures **(Mojiri *et al.*, 2019; Suresh *et al.*, 2024)**. *C. gariepinus* inhabit several aquatic settings, ranging from pristine to turbid, murky, and heavily contaminated water bodies, rendering it a valuable bioindicator for assessing pollution in freshwater systems **(El-Hak *et al.*, 2022)**.

Therefore, the current study aimed to quantify PAHs in sediment and assess their impact on the ovary of *C. gariepinus* collected from two examined locations as sentinel freshwater species.

MATERIALS AND METHODS

1. Sediment samples collection

Sediment samples (1kg each) were taken in triplicate from various points at site 1 and site 2 during July 2023, using an Ekman dredge bottom sampler from the canal bottoms concurrently with fish collection. Following sampling, the water was eliminated from the sediments using decantation, then transported in an ice box to the laboratory of the National

Research Center, Egypt. Samples were desiccated in darkness for 48 hours before to analysis **(Khedr *et al.*, 2023)**.

2. Quantification of 16 PAHs in sediment

High performance liquid chromatography (HPLC), specifically the Agilent 1260 series, was employed as the analytical and separation method to ascertain the amounts of 16 PAHs in sediment. The separation utilized a Zorbax Eclipse PAHs column (4.6mm x 150mm, 5μm). The mobile phase comprised water (A) and acetonitrile (B) at a flow rate of 2.0ml/ min. The injection volume measured 5μl. Temperature of column was sustained at 25°C. The PAH standards of the EPA 610 were acquired from Supelco, Bellefonte, PA, USA, for use in the laboratory of the National Research Center. Two grams of sediment samples were placed into a clean extraction container (50ml flask), followed by the addition of 20ml of acetone. The flask underwent ultra-sonication for 30 minutes. (Clifton ^{tm}, SW3H, UK) and purified process by solid phase extraction (SPE) with C18 mini-column cartridges. The mixes were agitated vigorously and subsequently decanted for thirty minutes **(Sarrazin *et al.*, 2006)**. Extracts were transferred to HPLC for fingerprint analysis using diode-array detection (DAD) and fluorescence detection (FLD) at 220nm.

3. Fish collection

Eight specimens of female catfish *C. gariepinus* were collected from various localities of two studied sites: four specimens from site 1 (total length 50–90cm and weight 1–3.7kg) and four specimens from site 2 (total length 45–80cm and weight 1.3–2.9kg), serving as the materials for this study conducted in July 2023. Trammel nets and basket traps (Gwabi) were the primary fishing gears employed to capture fish. Fish were analyzed in a fresh state and thereafter moved to the Zoology and Entomology laboratory, Zoology Department, Faculty of Science (Girls' Branch), Al-Azhar University, Nasr City, Cairo, Egypt for further testing. Fish were classified in the laboratory according to the classification of **Bishai and Khalil (1997)**. Total and standard lengths were quantified to the closest millimeter and documented. Fish were weighed to the nearest 0.1g, after which the further studies were conducted.

4. Sex hormone

The electrochemiluminescence immunoassay (ECLIA) is designed for application on Elecsys 2010, Modular Analytics E170, or (cobas e) immunoassay analyzers for the *in vitro* quantitative measurement of progesterone and estradiol in serum obtained from standard sampling tubes or tubes containing separating gel. The determination of progesterone was conducted following the methodologies established by **Runnebaum and Rabe (1994)** and **Wu (2006)**. The determination of estradiol (E2) was conducted following the methodologies established by **Johnson *et al.* (1993)** and **Melmed *et al.* (2015)**.

5. Statistical analysis

The data obtained from the HPLC analytical and ECLIA techniques were expressed as mean ± standard deviation and subjected to statistical analysis using a Student's T test (Levene’s test) utilizing the (Statistical Package for Social Sciences) (SPSS) (IBM SPSS Statistics Version 22; SPSS Inc., IL, USA) to compare the means.

6. Histopathological investigation

For histological analysis, anesthetized specimens were dissected, and the ovaries were excised and examined. The ovaries were sectioned into 5mm thick pieces and promptly fixed in alcoholic Bouin's fluid for a minimum of 48 hours, thereafter dehydrated in escalating concentrations of ethyl alcohol, cleaned in xylene, and embedded in paraplast wax (M.P.: 58°C). Transverse slices were prepared at a thickness of 4-6µm and stained with Harris's hematoxylin and eosin (H&E) stain using standard histological procedures **(Suvarna *et al.*, 2012)**. The stained slides were examined using a light microscope (XSZ-N107T) at various magnifications, subsequently photographed with a digital camera (Toup Cam, Ver. 3.7) and documented. This study was conducted to assess the effects of PAHs on ovarian tissues. Atretic oocytes were characterized based on histological differences, as outlined by **Gupta and Matti (1986)**.

RESULTS

1. Mean of 16 PAHs in sediment (mg/g)

The findings presented in Table (1) indicate that the measured values of PAHs (Naphthalene, Phenanthrene, chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)Pyrene, Dibenzo(a,h)anthracene and Benzo(g,h,i)Perylene) were higher in sediment from site 2 than site 1. In contrast, the measured values of pyrene, benz(a)anthracene, and indo(1,2,3-cd) pyrene were higher in sediment collected from site 1 than site 2. Fluorene value (1.006±1.743) was found only in sediment at site 2 (Table 1). Acenaphthylene, acenaphthene, anthracene and fluoranthene were entirely absent in sediment of two studied sites.

Statistical analysis

The mean concentrations of the measured PAHs in sediment were evaluated using a T-test (Table 1). The results showed no significant differences between the two investigated canals for most PAHs ($P > 0.05$), except for benzo(b)fluoranthene, which exhibited statistically significant differences between the two canals ($P < 0.05$).

2. Sex hormones

Data in Table (2) show the sex hormone in the serum of the blood of *C. gariepinus*; the mean value of progesterone (ng/ml) was higher in the serum of fish collected at site 1 (0.73±0.58ng/ ml) compared to site 2 (0.14±0.02ng/ ml). While, the mean value of estradiol (E_2) (pg/ml) were higher in serum of fish collected at site 2 than site 1, being 538.44±9.92ng/ ml and 266.77±107.18ng/ml, respectively.

Table 1. Mean ± SD of PAHs in sediment (mg/g) collected from two investigated sites

PAHs in sediment	Site 1	Site 2	Sig.
Naphthalene	0.210±0.011	0.284±0.065	NS
Acenaphthylene	0±0	0±0	-
Acenaphthene	0±0	0±0	-
Fluorene	0±0	1.006±1.743	NS
Phenanthrene	0.110±0.725	2.234±1.252	NS
Anthracene	0±0	0±0	-
Fluoranthene	0±0	0±0	-
Pyrene	1.483±1.618	0.798±1.383	NS
Benz(a)anthracene	0.386±0.375	0.209±0.363	NS
Chrysene	9.768±10.553	27.578±21.061	NS
Benzo(b)fluoranthene	0.213±0.220	1.720±0.748	*
Benzo(k)fluoranthene	3.306±1.412	4.665±2.170	NS
Benzo(a)Pyrene	2.608±2.505	5.152±2.841	NS
Dibenzo(a,h)anthracene	2.307±2.309	3.573±2.583	NS
Benzo(g,h,i)perylene	0.551±0.422	3.798±4.953	NS
Indo(1,2,3-cd) pyrene	0.376±0.651	0.172±0.298	NS

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

Table 2. Sex hormones (Mean ± SD) in serum of *C. gariepinus* collected from two investigated sites

Sex hormone	Site 1	Site 2
Progesterone (ng/ml)	0.726±0.578	0.136±0.020
Estradiol (E_2) (pg/ml)	266.68±107.181	538.438±9.924

3. Ovary of *C. gariepinus*

3.1. Morphology of ovary in C. gariepinus

The ovaries in *C. gariepinus* have paired, elongated structures that are round in cross-section. The morphology of the ovary in *C. gariepinus* specimens obtained from site 1 exhibited normal morphology. The ovarian epithelium was discerned by the presence of a transparent fluid beneath the ovary, and the oocytes had a yellowish hue (Fig. 1). The ovarian morphology in *C. gariepinus* specimens taken from site 2 exhibited moderate abnormalities. The presence of a brownish fluid beneath the ovarian epithelium was seen, whereas the oocytes exhibited a reddish hue (Fig. 2).

Fig. 1. Photograph of the ovary of *C. gariepinus* collected from site 1

Fig. 2. Photograph of ovary of *C. gariepinus* collected from site 2

3.2. Relation between oocyte maturation and atresia in ovary of C. gariepinus

Data illustrated in Fig. (3) indicate that the largest percentage of mature oocytes, comprising (50.43%) of the total oocyte count, was seen in the ovaries of *C. gariepinus* at site 1, while the lowest (20%) was recorded in the ovaries of fish at site 2. The proportion of immature oocytes was minimal, comprising 7.22% of the total oocytes in the ovaries of fish at site 1 and 5% in the ovaries of fish at site 2. The minimum proportion of atretic oocytes (42.35%) of the total oocyte count was seen in the ovaries of fish from site 1, while the maximum (75%) was noted in the ovaries of fish from site 2.

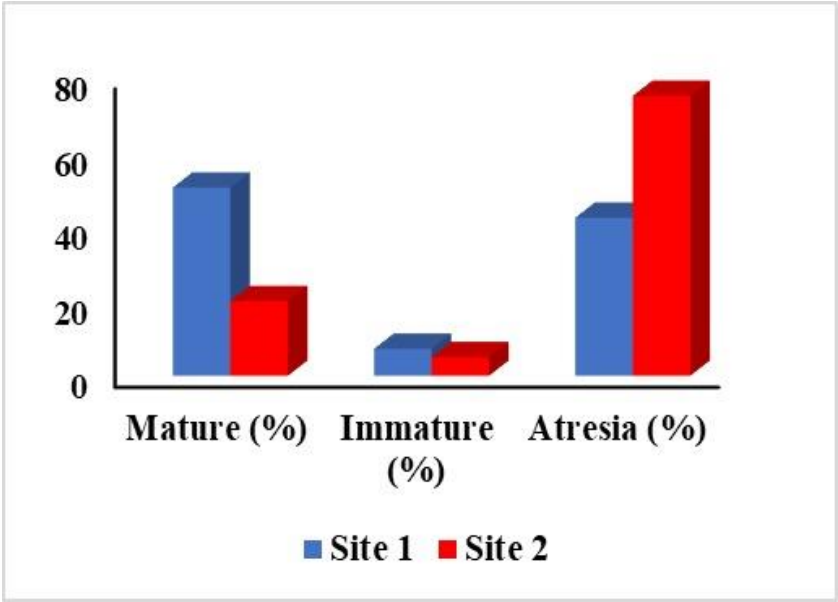

Fig. 3. Percentage of maturation and atresia (%) in ovaries of *C. gariepinus* collected from two studied sites

3.3. Histopathology of ovary

The histological characteristics of the ovary in *C. gariepinus* specimens obtained from site 1 exhibited normal morphology. The predominant component of mature oocytes was the pre-spawning stage (50.43%), with minor proportions of immature oocytes (7.22%) and atretic oocytes (42.35%) (Fig. 4A).

The oocytes included huge, intensely eosinophilic yolk globules that encroach upon the area occupied by lipoid droplets. The yolk globules were distributed uniformly throughout the ooplasm. The oil droplets coalesced and diminished in quantity. The nuclei, referred to as "germinal vesicles," possess uneven contours. The nucleoli were less defined and seemed dispersed inside the nucleoplasm. The zona radiata appeared more substantial (Fig. 4B).

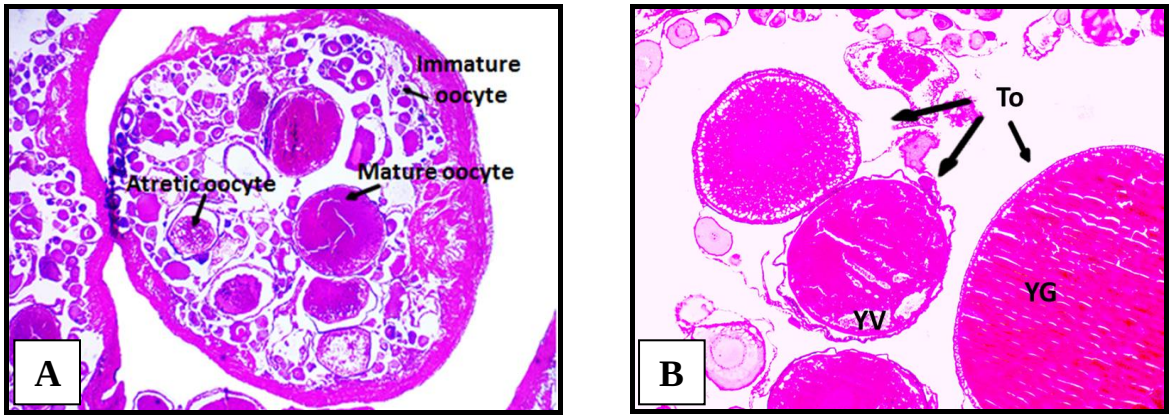

Fig. 4. Photomicrograph of T.S. in the ovary of *C. gariepinus* obtained from site 1, showing: **A.** Normal structure of the mature oocytes and some of immature and atretic oocytes (H&E 40 x); **B.** Tertiary oocytes (TO) containing yolk vesicles (YV) and yolk granules (YG) (H&E 100 x)

The histological characteristics of the ovary in *C. gariepinus* specimens obtained from site 2 exhibited atresia. The composition of oocytes consisted of mature oocytes (20%). A small proportion (5%) of immature oocytes was identified, while around 75% of the total oocyte count exhibited various characteristics of atresia (Fig. 5A).

The histological analysis of the ovary in *C. gariepinus* specimens obtained from site 2 exhibited several histological alterations. Microscopic examinations revealed degenerative alterations (atresia) in certain oocytes and proliferative modifications in the granulosa of other oocytes, leading to the adherence of their cellular covering. Furthermore, certain oocytes collapsed and exhibited aberrant irregularities in form. Furthermore, the detachment of the follicular layers from the oocytes was noted (Fig. 5B).

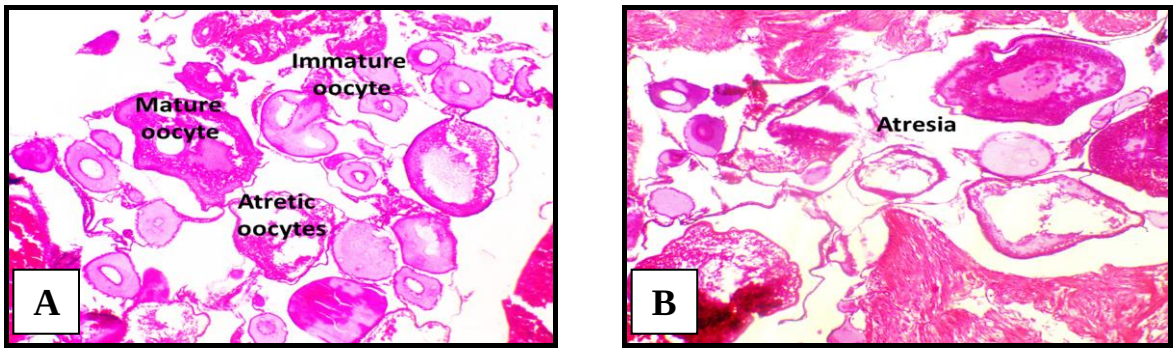

Fig. 5. Photomicrograph of T.S. in the ovary of *C. gariepinus* obtained from site 2, showing: **A.** normal structure of mature oocytes, immature oocytes, and atretic folicles (H&E 40 x); **B.** different atretic follicles (H&E 100 x)

4. Atresia in ovary of *Clarias gariepinus*

Histological examination of ovary *C. gariepinus* collected from two studied sites indicated that the atretic oocytes originate from either vitellogenic or mature oocytes that failed to progress further, subsequently undergoing degeneration and reabsorption in the examined species (Fig. 6A). Follicular atresia was observed as follows: the follicular epithelium transitions from squamous to columnar, exhibiting unclear cell borders and rounded nuclei. The striation of the zona radiata of the oocyte has vanished. Initially, solitary ruptures manifested, followed by a rapid fragmentation into discrete pieces. The follicular epithelial cells are apically indented with the oocyte and plainly serve as phagocytes in this context. The follicular phagocytic cells infiltrate the yolk components of the oocytes. The yolk components display evident symptoms of distortion, and the nuclei of the oocytes vanish. The phagocytic cells, upon invading the oocyte, initially targeted the proteins, whereas the carbohydrates and lipids remained encapsulated inside the theca enclosing the oocytes until the reabsorption process was completed (Fig. 6B).

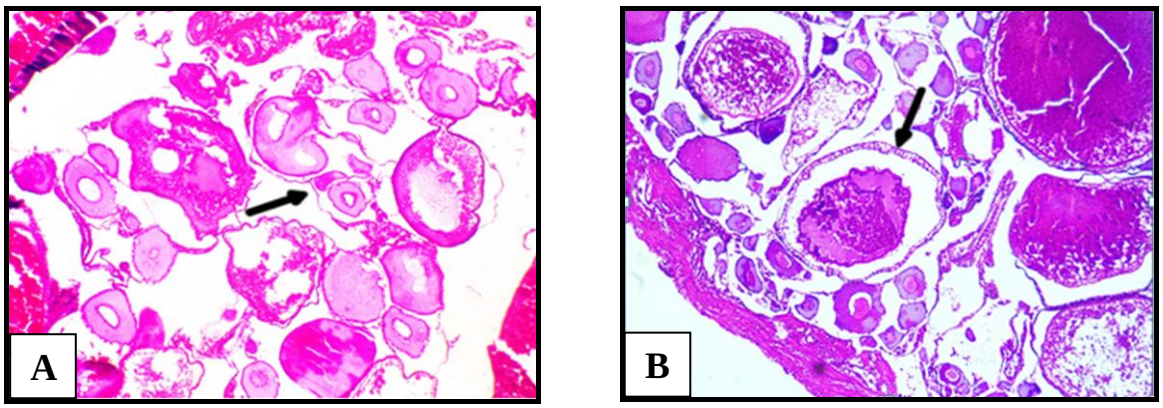

Fig. 6. Photomicrograph of T.S. in the ovary of *C. gariepinus* collected from: **A.** Site 2 showing degenerated oocytes (arrow) (H&E 400 x); **B.** Site 1 showing the follicular epithelium is columnar pattern (arrow) (H& E 100 x)

Atretic oocytes can be categorized into two primary kinds based on histological distinctions: non-bursting atresia and bursting atresia.

4.1. Non-bursting atresia

A particular type of atresia was prevalent in early oocytes. This atresia is characterized by an intact follicular wall and can be categorized into four types:

Capsulated atresia: This type is characterized by a significant decrease in ooplasm size, which appears as a dark-stained mass. The cells of the stratified epithelium proliferate and penetrate the liquid yolk below as the atresia process progresses. As atresia advances, the entire yolk mass disintegrates and merges with the follicular cells that encroach on the region. Additionally, the theca folliculi thickens and becomes significantly more vascularized. The atretic follicle loses its vascularity, resembling a vacant sac that is eventually absorbed by the ovarian stroma during the late phase of atresia (Fig. 7A).

Lipoidal atresia: In this type, the follicular wall appears relatively thick and convoluted. The ooplasm contains vacuoles that may harbor lipoid compounds. During late lipoidal atresia, the oocyte membrane exhibits thickening and wrinkling (Fig. 7B).

Cystic atresia: In cystic atresia, the oocyte decreases in size and loses its characteristic identity. This results in a substantial, transparent pre-vitelline gap between the ooplasm and the oocyte membrane (Fig. 7C).

Nuclear atresia: In this type, a large, clear lunar space forms inside the nuclear membrane due to the contraction and loss of normal nuclear substance characteristics (Fig. 7D).

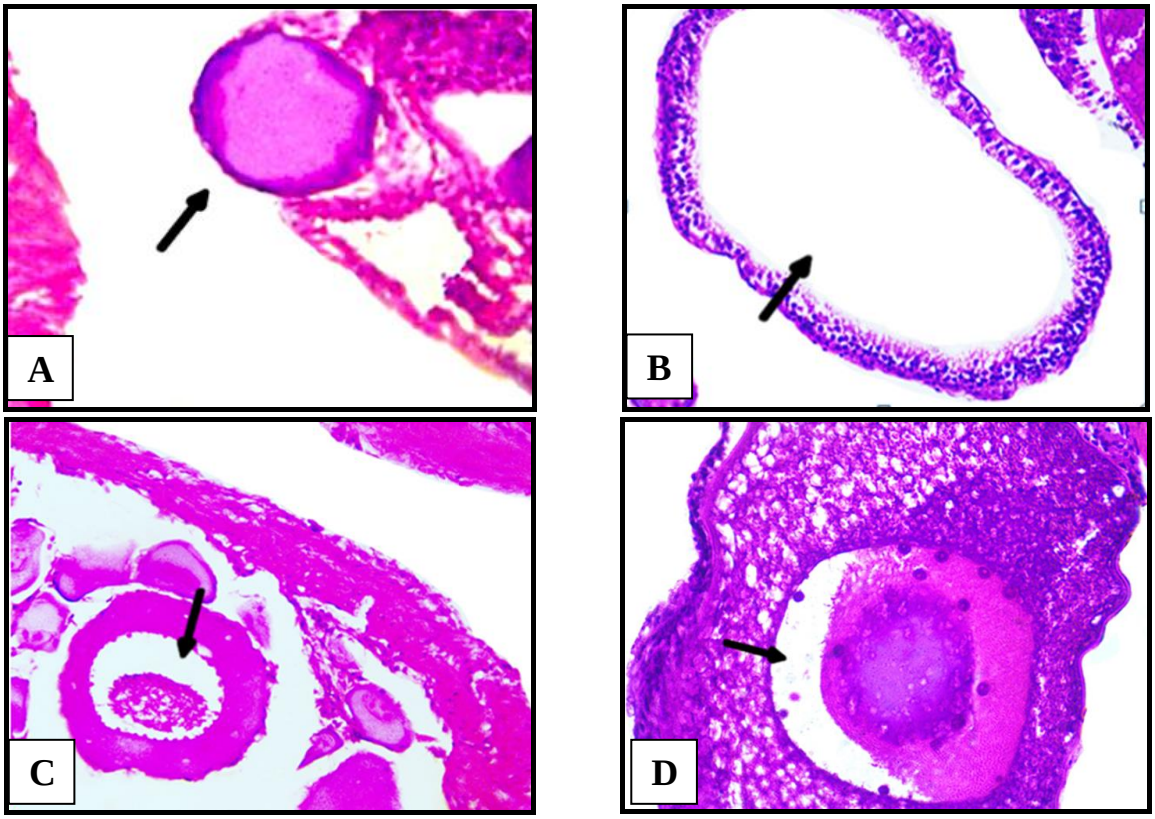

Fig. 7. Magnified section of T.S. in the ovary of *C. gariepinus* collected from two investigated sites showing types of non-bursting atresia, **A.** Capsulated atresia (H&E 400 x); **B.** Lipoidal atresia (H&E 400 x); **C.** Cystic atresia (H&E 400 x); **D.** Nuclear atresia (H&E 400 x)

4.2. Bursting atresia

This form was observed in late-developing oocytes. It is defined by a breached follicular wall and can be categorized into five types:

Multiple bursts: In this type, atretic follicles are seen in multiple follicular regions. The oocyte membrane is thicker than normal (Fig. 8A).

Individual bursts: Atretic oocytes display a singular bursting point, leading to the extrusion of follicular contents into the stroma. The wall of the atretic oocyte appears robust (Fig. 8B).

Liquefied bursts: Atretic follicles of this kind contain large vacuoles in the ooplasm, and the oocyte wall thickens and becomes wrinkled in the later stages (Fig. 8C).

Phagocytic bursts: In this variant, follicular cells transform into phagocytic cells that infiltrate the ooplasm through disrupted areas of the follicular wall. These cells show a reduction and gradual decline (Fig. 8D).

Advanced phagocytic bursts: Phagocytic cells penetrate the ooplasm through breached sections of the follicular wall, dividing the oocyte into two or three smaller fragments, which leads to degeneration (Fig. 8E).

Ultimately, all types of atretic oocytes undergo degeneration and disappear within the stroma.

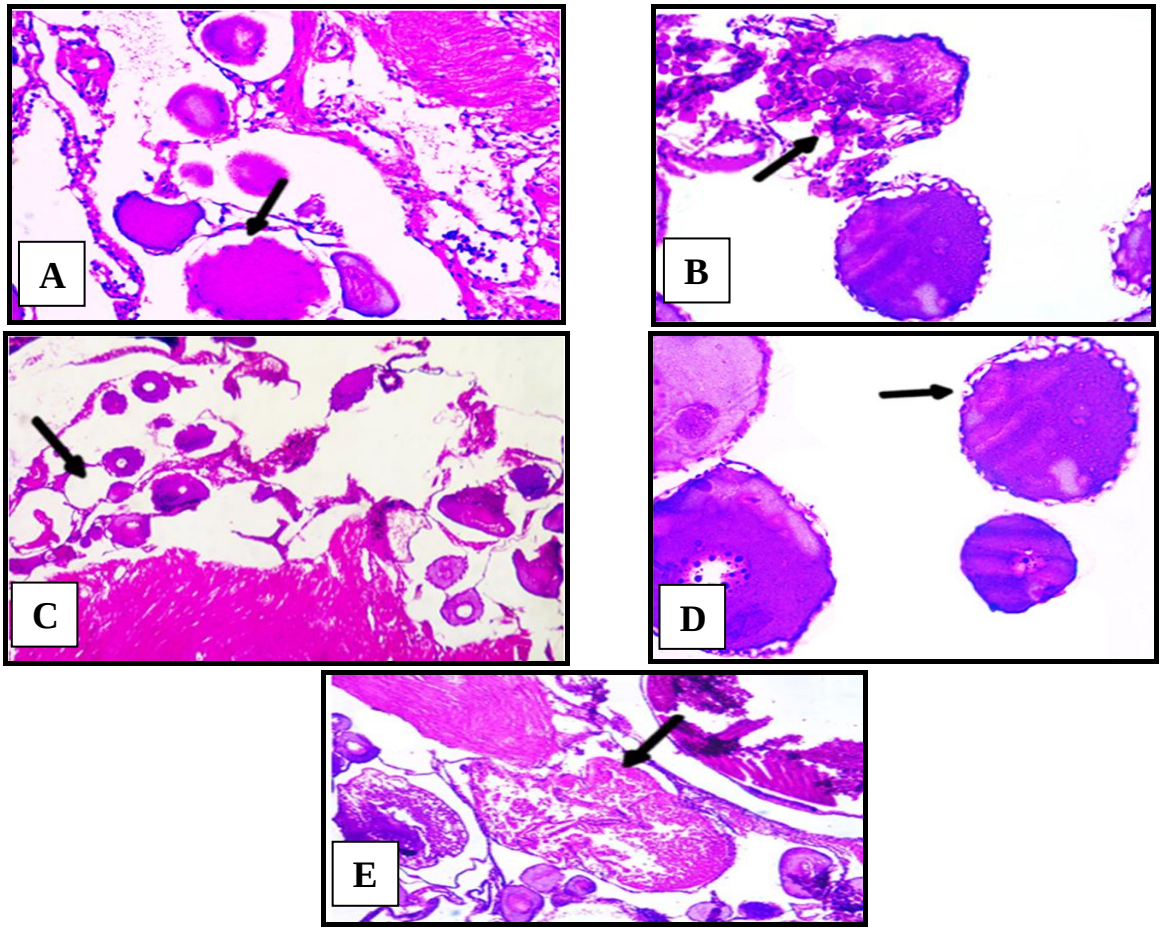

Fig. 8. Magnified section of T.S. in the ovary of *C. gariepinus* collected from two investigated sites showing types of bursting atresia: **A.** Multiple bursts (H&E 100 x); **B.** Single bursts (H&E 400 x); **C.** Liquified bursts (H&E 100 x); **D.** Phagocytic bursts (H&E 400 x); **E.** Advanced phagocytic (H&E100 x)

DISCUSSION

Due to their physical and chemical properties, PAHs usually move from the water column into the sediment in aquatic environments. High molecular weight (HMW) PAHs, which consist of multiple aromatic rings, exhibit an increased propensity to be dispersed by water currents, and eventually become assimilated with the sediment by quickly adsorbing and accumulating onto sediment particles **(Tehrani *et al.*, 2013; Adeniji *et al.*, 2018; Dai *et al.*, 2022; Montuori *et al.*, 2022; Younis *et al.*, 2023)**. In line with **Wu *et al.* (2019)**, soil PAH contamination is categorized into three classifications: non polluted (PAHs < 200ng. g^{-1}); slightly polluted (PAHs 200–600ng. g^{-1}); and substantially polluted (PAHs > 1,000ng. g^{-1}).

The total 16 PAHs in sediment were higher at site 2 compared to site 1. The primary source of pollution may stem from the waste sediment discharged by water and sewage treatment facilities in Kafr El-Khadra Village at the initial point of site 2. Additionally, pollution is contributed by the ceramic and marble factory in Tilwana Village, urban development, and the improper disposal of refuse into El-Bahr El-Pharaouny Canal. The concentration of 16 PAHs in sediment at Bahr Shebeen Canal is 66.95mg/ L, indicating a significant reduction compared to the findings of **Nasr *et al.* (2010)**, which reported total concentrations of 13 PAHs in sediment ranging from 119.777mg/ g at Bahr Shebeen Canal to 270.155mg/ g at El-Embaby Drain. Regarding the composition of individual PAHs in sediment, the majority of investigated compounds were identified at all locations. The quantities of PAHs in sediment samples are many orders of magnitude greater than those in the aqueous phase, as degradation rates are often slow, mainly due to the insufficient oxygen necessary for initiating ring breakage **(Cardellicchio *et al.*, 2007)**.

In this study, the composition of PAHs in sediment were predominantly characterized by four-ring compounds. The variance in contaminant prevalence among different PAHs may be ascribed to those PAHs capable of enduring down-column movement and reaching the sediment bed; such PAHs are expected to possess a relatively (HMW), rendering them more resistant to breakdown processes. Furthermore, processes like biodegradation targeted PAHs in sediments, resulting in the persistence of those PAHs that were resistant to degradation. Conversely, chrysene ($C_{18}H_{12}$) exhibits a much higher concentration than other individual PAHs at the two locations, attributable to its greater stability and less susceptibility to degradation in comparison to lower molecular weight PAHs. This stability enabled prolonged environmental persistence and accumulation in sediments, where it adheres firmly to particles, resulting in elevated concentrations in sediments relative to water or soil **(Li *et al.*, 2023; Qi *et al.*, 2024)**.

On the other hand, the findings indicated that the mean values of all PAHs in the sediment exhibited no significant differences except benzo(b)fluoranthene gave statistically significant differences between the two investigated canals, possibly due to differences in local sources of pollution such as industrial discharges, urban runoff, or agricultural activities, might be contributing varying amounts of benzo(b)fluoranthene to each canal, environmental conditions like water flow, sediment composition, and microbial

activity can influence the degradation and accumulation of PAHs, including benzo(b)fluoranthene or biological factors affecting its distribution and metabolism. Benzo(b)fluoranthene might have unique chemical properties that make it more prone to accumulation or less susceptible to degradation in one canal compared to the other **(Xiu *et al.,* 2014)**.

Numerous aquatic pollutants induce reproductive harm in fish **(Barber *et al.,* 2012; Liu *et al.,* 2018; Marlatt *et al.,* 2022)** and may change gonadal histopathology, vitellogenin concentrations, gonadotropin levels, and sex steroid hormones. Aquatic contaminants can impact these characteristics variably between species and their reproductive strategies **(Segner, 2011)**. Pollutants adversely affect the endocrine system, inhibiting hormone production and disrupting the development of gonads and gametes **(Mansour *et al.,* 2018; Shahid *et al.,* 2022)**. Therefore, the serum levels of sex hormones in *C. gariepinus* in this study showed the mean concentration of progesterone (P4) (ng/ml) was greater in the serum of fish obtained from site 1 compared to site 2. Furthermore, PAHs adversely impacted the reproductive organs, diminishing their hormone production capacity. The mean estradiol (E2) concentration (pg/ml) in the serum of the catfish from site 2 is unexpectedly higher than that from site 1, attributed to PAHs stimulating the activity of aromatase which is an enzyme that converts androgens, such as testosterone, into estrogens including estradiol. Elevated aromatase activity may lead to augmented estradiol levels **(Barron *et al.,* 2004)**. PAHs induced oxidative stress, resulting in cellular and tissue damage, including that of hormone-producing cells. Furthermore, PAHs, recognized as endocrine disruptors, disturb the normal functioning of the endocrine system, they can bind to estrogen receptors, leading to altered behavior and variations in sex hormone levels, including estradiol **(Scholz & Klüver, 2009; Zhong *et al.,* 2017)**. PAHs can induce the production of vitellogenin, a yolk precursor protein typically found in females' fish that is lead to reproductive anomalies **(Arukwe & Goksøyr, 2003)**.

Similar to the current study, reproductive hormones were also altered in the catfish subjected to pyrogallol treatment. **Hamed *et al.* (2024a)** noted that after 15 days of exposure to 1, 5, or 10mg/ L pyrogallol, the African catfish exhibited elevated levels of LH and reduced levels of FSH compared to controls. The plasma estradiol regulates pituitary LH levels via a positive feedback mechanism **(Dickey & Swanson, 1998)**. This result aligns with findings on other aquatic pollutants, indicating that ethinyl estradiol disrupts the reproductive hierarchies of zebrafish **(Coe *et al.,* 2008)** and diminishes sex steroid levels in fathead minnows (*Pimephales promelas*) **(Salierno & Kane, 2009)**. In contrast to our findings, **Ganguly *et al.* (2023)** observed reductions in serum E2 and FSH in *Labeo catla* subjected to cypermethrin exposure. In tilapia subjected to plastic pollutants for a duration of 14 days, a decrease in E2 and testosterone levels, together with a reduction in spermatogenic cell count, has been documented **(Hayati *et al.,* 2022)**. **Monteiro *et al.* (2000)** found decrease in plasma 17 β-estradiol due to chronic effects of PAHs on female flounder (*Platichthys flesus*). Collectively, there is substantial data indicating that reproductive damage is attributable to several environmental pollutants.

According to **Getnet *et al.* (2024)**, atresia, defined by nuclear disintegration, breakdown of the vitelline membrane, proliferation and enlargement of follicular cells, and liquefaction of yolk globules, is linked to exposure to ecological contaminants. Atresia in vitellogenic oocytes, observed under both natural and experimental conditions, has been identified as a significant predictor of pathological states in fish exposed to pollutants **(Corriero *et al.*, 2021)**. This study examines the morphology of ovary *C. gariepinus* specimens obtained from the two studied sites.

Ovarian atresia samples from low and high quantities of PAHs in the water and sediment were obtained from site 1 and site 2, respectively. Atresia in female fish arises alterations in ovarian morphology due to contaminated water. This finding was documented in various fish species recording increased pesticide levels in *Colisa lalia* **(Sukumar & Karpaganapathy, 1992)**; cyanide compounds in *Tilapia zillii*, *Clarias lazera* and *Chrysichthys reuppelli* **(Alne-Na-Ei, 1997)**; sediment contamination in *Chrysichthys rueppelli*, **(Alne-Na-Ei & Rady, 1998)**; chronic dietary exposure to single PAHs was studied in flounder (*Platichthys flesus*) **(Monteiro *et al.*, 2000)**; pollutants or global warming in *Chalcalburnus tarichi* **(Ünal *et al.*, 2007)**; in some Mediterranean Sea fishes from the Egyptian coast by long spawning season in *Terapon puta* and *Lithognathus mormyrus* **(Khalaf-Allah & Shehata 2011)**, and heavy metals in *Tilapia zillii* **(Azab *et al.*, 2019)**.

This study documented the lowest percentage of mature oocytes at site 1, whereas the largest percentage of atretic oocytes was observed in the ovaries of fish from site 2. This indicates that the maturation of oocytes diminishes while atresia escalates with elevated levels of PAHs. The disruption of ovarian functioning in fish exposed to PAHs in water and sediment alter the expression of genes implicated in oocyte maturation and apoptosis. This may lead to the suppression of gene expression necessary for oocyte development and the upregulation of genes that facilitate atresia **(Cousin & Cachot, 2014)**. Furthermore, PAHs interfered with cellular signaling pathways that governed oocyte maturation. Therefore, **Cousin and Cachot (2014)** detected that PAHs activated the aryl hydrocarbon receptor (AhR) pathway, resulting in the activation of detoxifying enzymes and other molecular cascaded that disrupt normal cellular functions. **Mazrouch and Mahmoud (2009)** indicated that the gonads of *Oreochromis niloticus* from the Rosetta Branch exposed to elevated pollution concentrations had a greater prevalence of gonadal abnormalities. **Amer and Ahmed (2019)** demonstrated that the impact of water contaminants on the gonadal structure of the wild tilapia (*O. niloticus)* leads to an increased proportion of atretic follicles and the reabsorption of vitellogenic oocytes. **Weber and Janz (2001)** investigated the effects of PAHs on the juvenile channel catfish (*Ictalurus punctatus*). Their study demonstrated a significant increase in ovarian cell apoptosis in the exposed fish, highlighting the detrimental impact of PAHs on reproductive health.

Genarally, this study suggests that the cessation of development in certain ovarian regions and the advanced atresia observed in fish ovaries from site 2 may result from PAH contamination and its detrimental impact on fish sex hormones. This may be linked to the consequences of agricultural, industrial, and sewage waste discharge into the site 2.

Histological alterations in fish gonads due stressors in aquatic environments serve as an indicator for aiding the bio-monitoring of aquatic ecosystems **(Ghamdi *et al.,* 2014)**. While, the current findings detail the histological characteristics of ovary *C. gariepinus* specimens obtained from site 1 exhibited minimal atresia. The poor atresia may be attributed to the contaminating effects of PAHs resulting from urban development, including dish and laundry washing, animal cleaning, and the disposal of certain waste. The histological changes found in the ovaries are consistent with those reported by **Mohamed (2003)** and **Hamed *et al.* (2024b)**. Furthermore, histological alterations in the ovary of *C. gariepinus* exhibited an increased damage correlating with elevated amounts of PAHs at site 2. Previous studies indicated that pollutants could alter the gonadal histological architecture in fish, aligning with the present results. *Catla catla* collected from polluted environments exhibited signs of hypogonadism, dysgenesis, pronounced hypergonadism, regional necrosis and changes, as well as gonadal cell degeneration **(Bashir *et al.,* 2022)**. The ovary of tilapia (*Saratherodon galilaeus*) obtained from a polluted canal exhibited enlarged vitellogenic regions containing substantial debris and degraded immature oocytes **(Getnet *et al.,* 2024)**.

Atresia transpires when eggs cease development, do not ovulate from the ovary, and are subsequently reabsorbed into the gonad **(Arocha, 2002)**. The current study's histological analysis of fish ovaries from the two locations revealed that atretic oocytes may be categorized into two primary types: non bursting and bursting atresia. **Kamel (1990)** categorized atretic follicles based on their sizes; however, the present study's classification relied on histological descriptions. Identical results were achieved by **Ramadan and El-Halfawy (2007)**, **Khalaf-Allah and Shehata (2011)**, and **Azab *et al.* (2019)**. They stated that the distortion of the oocyte wall is regarded as the initial phase of atresia, followed by the phagocytosis of oocytes. The ongoing study revealed that non bursting atresia was prevalent in the early oocytes. It is distinguished by the intact follicular wall and can be categorized into four types: nuclear, capsulated, lipoidal, and cystic atresia. Only three forms (capsulated, lipoidal, and cystic atresia) were identified by **Ramadan and El-Halfawy (2007)**, **Khalaf-Allah and Shehata (2011)** and **Azab *et al.* (2019)**. Our study relies on histological examination to indicate nuclear atresia. The current investigation examined the impact of PAHs on the ovaries of *C. gariepinus*, noting a bursting atresia in late-developing oocytes. This type of atresia is characterized by a breached follicular wall and can be categorized into five types: numerous bursts, single bursts, liquid bursts, phagocytic bursts, and advanced phagocytic bursts. **Azab *et al.* (2019)** observed similar results, while **Ramadan and El-Halfawy (2007)** and **Khalaf-Allah and Shehata (2011)** documented only four types. Atresia is prevalent in the later stages of the maturation process and is linked to significant energy depletion and environmental conditions **(Kurita *et al.,* 2003; Ramadan & El-Halfawy, 2007)**.

CONCLUSION

The potential impact of PAH exposure on fish reproduction is crucial. The current study indicates that PAHs may interfere with sex steroid hormones, leading to reproductive impairments in *C. gariepinus* by affecting ovarian function. We identified various

histopathological degradations in the ovaries of *C. gariepinus* due to exposure to waterborne PAHs and their accumulation in sediment. Our findings suggest a significant alteration in the recruitment efficacy of the fish population. Further characterization of the pathways involved is necessary to develop more sophisticated biomarkers, and we propose that the catfish could be a valuable resource for this purpose. Therefore, agricultural, industrial, and sewage discharges into freshwater bodies should undergo technical treatment to protect aquatic life and natural resources.

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