

## Utilization of birds of different mating system behavior and feeding habits as a bioindicator for urbanization lead pollution

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### ABSTRACT

Anthropogenic contamination is spreading through every environment on the planet that affects the whole biota. Negative effects have been observed recently in a number of taxa, including birds. Birds are crucial as bioindicators as one of the best studied groups of organisms because they are noticeable, relatively simple to observe, and the subject of public interest and concern. Therefore, evaluation of lead (Pb) levels in the liver, muscle, kidney, and feathers of laughing dove (*Streptopelia senegalensis*), Egyptian barn swallow (*Hirundo rustica savignii*), and house sparrow (*Passer domesticus niloticus*) was done. These bird species, which live in the coastal region of Damietta, have various feeding routines, habitat preferences, and mating strategies. In both the breeding and nonbreeding seasons, these wild birds were collected. Measurement of the levels of lead (Pb) in their liver, kidney, muscle, and feathers were evaluated. The results showed that females of laughing doves and barn swallows had significantly higher Pb concentrations than males, while males of house sparrows accumulated significantly higher Pb concentrations than females. This could be attributed to the active food search in both female laughing doves and swallows, and male sparrows. House sparrows, with the widest range of distribution near main roads, showed the highest concentration of Pb, especially in the liver, kidney, and feather than laughing doves and swallows, which prefer the wild rural areas. Histological examination of the gonads revealed unhealthy testes and ovaries. The ovarian tissue was less impacted than the testicular tissue, especially during the nonbreeding season. Compared to the dove and swallow, the sparrow's gonadal architectural histopathological findings were more noticeable. It seems that testes are more affected and more related to the results of lead measurements, and can be used as a bioindicator for heavy metal pollution. The findings offer also some proof of the house sparrow's potential as a biomonitor for urban heavy metal pollution. This research emphasises the significance of using biomonitors from various habitat types and feeding guilds.

**Keywords:** Egyptian barn swallow; House sparrow; Laughing dove; Pb contamination.

### INTRODUCTION

Expansion of industrial activities and urbanization contaminate the ecosystem with a huge load of pollutants. Metals are one of the main threats to wildlife and human health as well, especially their ability to be biomagnified through the food chain (Janadeleh and Kameli, 2017; Ali and Khan, 2019).

Lead (Pb) is present naturally in the Earth's crust in small amounts, but its distribution increased in the environment due to anthropogenic activities, industrial expansion, and urbanization (Levin *et al.*, 2008). Industrial and urban soils are often rich in Pb at levels of micrograms per gram in urban areas (Kabata-Pendias, 2010). Consistent with this, wildlife living in industrialized, urbanized, and intensively agricultural areas has been reported to have elevated trace metal concentrations. For example, urban sparrow, starling, and pigeon populations had higher concentrations of heavy metals than rural populations (Bichet *et al.*, 2013; Kekkonen *et al.*, 2012; Millaku *et al.*, 2015; Nam and Lee, 2006; Swaileh and Sansur, 2006). Several symptoms of lead (Pb) poisoning and toxicity in birds have been identified (Swaileh and Sansur, 2006), primarily in specimens with the highest lead exposure. Symptoms include increased kidney weight, altered mitochondrial structure and function, and the presence

of renal intranuclear inclusions (Zaman *et al.*, 2022; Sato *et al.* 2022)

Animals are often used as predictors of ecosystem pollution because they are constantly exposed to contaminants from the environment (Clark *et al.*, 1988). Birds are considered an ideal model to monitor environmental pollution like heavy metals contamination, as they occupy a wide range of trophic levels in different food chains, Due to their relatively long lifespan, they tend to accumulate contamination. (Furness *et al.*, 1993; Becker *et al.*, 2003; Zhang and Ma, 2011). Exposure to heavy metals can vary by species' diet and their position in the food web. Birds at higher trophic levels tend to accumulate higher concentrations of heavy metals (Zhilong *et al.*, 2017; Einoder *et al.*, 2018). Heavy metals have been shown to inhibit the immunity and endocrine functions of birds, cause reproductive system disorders, increase susceptibility to disease and stress, and alter behavioral patterns. These severe abnormalities affect the survival and reproduction of birds (Scheuhammer, 1987; Bernanke and Kohler, 2008). Birds accumulate high levels of pollutants in their tissues and are particularly susceptible to physiological effects. Therefore, certain urban bird species have been proposed as bioindicators of urban heavy metal pollution (Bichet *et al.*, 2013; Nam and Lee, 2006; Swaileh and Sansur, 2006).

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The objective of the current study is to examine what extant birds with different feeding habits, habitat distribution ranges, and reproductive mating system behavior, can be at risk of environmental pollution. Lead (Pb) was evaluated in three of the most widely distributed birds in Egypt: the house sparrow, Egyptian barn swallow, and laughing dove. These feral resident birds have been chosen because they are appropriate as bioindicators to biomonitor environmental lead pollution since they are resident, not migratory, primarily associated with the urban environment, and their basic niche is characterised by interaction with human activities such as roosting in human structures, exploiting open garbage for foraging, and existing in the same spot with humans without disturbance. The occupancy of this wide range reflects the tolerance to environmental stress and high reproductive rate (Swaileh and Sansurb, 2005).

Therefore, in order to achieve our aim, we looked for the Pb concentration among genders of the same species to test if the difference between males and females' behavior affects the accumulation within the same species. The results were then compared for the three bird species caught from the same habitat to test which species could be useful as a bioindicator for Pb pollution. Exposure to heavy metals causes adverse effects on the reproductive system; therefore, ovaries and testes were subjected to histological examination to investigate the degree of damage.

## MATERIALS AND METHODS

### Sample collections

Birds were captured using mist nets in the coastal area of Damietta Governorate. The coastal area is dominated by anthropogenic activities including industries, farming, and gas liquefaction. All of these are considered sources of pollution, although they form an important habitat for both migratory and resident feral birds. The unique location of the coastal area in Damietta as a part of Delta with highly diverse agriculture activity and plantation beside the coastal sand bar makes it a suitable habitat for many migratory aquatic and passerine birds. The nature of Delta as main habitat type is the agricultural land making it suitable for feral resident birds foraging, providing nest material, and roosting.

Birds were captured on several occasions from a coastal area during breeding (March to June) and non-breeding seasons (July to September) for different foraging activity patterns. Three bird species were selected for this study according to different foraging activities and differences in mating systems, the bird species chosen were the laughing dove, Egyptian barn swallow, and house sparrow. The total samples were (n=36, 12 for each species). Samples of kidney, liver, muscle and feathers from tail, chest, and wing were stored at -20 °C.

### Morphometric measurements

Six morphometric traits were measured which are BW: Body Weight, H: Head length, BL: Bill Length,

TA: Tarsus, W: Wing, and TL: Tail. Laughing doves showed significant differences between males and females in BL, W, and TL.

### Lead (Pb) estimation

Tissue samples were homogenized using a mortar, then 1g was weighed and transferred to 50 mL conical flasks. For digestion, samples were soaked overnight in a mixture of 10 ml (HNO<sub>3</sub>/HClO<sub>4</sub>, 9:1) and then digested at 70°C. After digestion, samples were diluted to 50 mL with deionized water and the solutions were filtered using filter paper. The concentrations of Pb in the kidney, muscle, liver, and feather were determined by the Flame Atomic Absorption Spectrophotometer. Samples were measured in duplicates; blank and standard samples were analyzed for all samples. The results were expressed as mg kg<sup>-1</sup> (Sheta *et al.*, 2019).

### Histology

In both the non-breeding and breeding seasons, the left ovary and testes were sectioned after fixation. Tissue samples were dehydrated in ascending concentrations of ethyl alcohol (70, 90, and 100%), cleaned in xylol, and then embedded in paraffin wax at 60 °C. A rotary microtome was used to cut transverse sections at 5 microns in thickness and mount them on slides. The sections were dewaxed in xylene, hydrated in descending grades of ethyl alcohol, stained with Hematoxylin and Eosin (H&E), washed, dehydrated, clarified, mounted with Canada balsam, and covered for light microscopy examination.

### Statistical Analysis

Statistical package programs of SPSS, version 19, were used to analyze the data statistically. One-way analysis of variance (ANOVA) was applied. A t-test was applied to determine the statistical difference between the two groups at significant level of  $p \leq 0.05$ .

## RESULTS

### Morphometric measurements

Based on the morphometric measures, which are shown in Table (1); tail. Laughing doves showed significant differences between males and females in BL, W, and TL. Both swallow and house sparrows showed no significant differences between males and females except in BW.

### Lead levels in the selected tissues

Females and males revealed a different affinity to accumulate Pb from the environment. Comparisons of mean values of Pb concentrations in liver, muscle, kidney, and feather between males and females of laughing dove, swallow, and house sparrow were done (Fig. 1). The results demonstrate that Pb concentrations in many organs differ significantly between males and females. Overall, it was shown that female laughing doves and swallows had larger Pb accumulations in several organs than corresponding males, in contrast to male house sparrows, which had higher Pb concentrations than females. *S. senegalensis* showed a significant difference ( $p \leq 0.05$ ) in Pb concentration in kidneys and feathers. In males, house sparrows were significantly higher in Pb concentrations in the liver,

**Table (1):** Morphometric comparisons between males and females in birds under study.

Measured parameters	Studied Birds								
	<i>Streptopelia senegalensis</i> (Laughing Dove)			<i>Hirundo rustica savignii</i> (Barn swallows)			<i>Passer domesticus niloticus</i> (House Sparrow)		
	Male	Female	P	Male	Female	P	Male	Female	P
Body weight (g)	98.23±4.33	92.53±4.49	0.08	12.55±0.47	14.87±1.06	0.02*	24.23±0.55	21.47±0.66	0.01*
Height (cm)	4.88±0.11	4.64±0.08	0.05*	3.21±0.07	3.27±0.04	0.24	3.66±0.05	3.56±0.06	0.12
Bill length (cm)	1.48±0.02	1.41±0.03	0.04*	0.57±0.03	0.58±0.03	0.39	0.99±0.03	0.96±0.04	0.31
Tarsus (cm)	1.75±0.06	1.74±0.06	0.47	0.75±0.05	0.69±0.04	0.17	1.67±0.06	1.6±0.04	0.21
Wing Length (cm)	13.09±0.14	12.46±0.2	0.01*	11.33±0.15	11.2±0.17	0.15	7.85±0.16	7.41±0.2	0.05*
Tail Length (cm)	12.42±0.30	11.67±0.23	0.03*	8.05±0.54	7.74±0.35	0.33	5.5±0.11	5.3±0.09	0.10

Data with asterisks, for each species, are significantly different at  $p \leq 0.05$ .

kidney, and feathers (Fig. 1). However, Barn swallow organs of the females had considerably higher Pb contents across the board than those from male. Meanwhile, the evaluated amounts of Pb in the liver, kidney, and feathers of house sparrow males were significantly greater ( $p \leq 0.05$ ).

Pb concentrations in various organs of the examined birds were evaluated (Fig. 2). The result demonstrated that house sparrow, compared to laughing doves and swallows, had significantly higher Pb concentration in their liver, kidney, and feathers. Meanwhile, laughing dove muscles exhibited more Pb concentration than swallows and house sparrow muscles.

#### Histopathology of the gonads in the studied species

##### Testis

Although birds have been wildy collected, several histopathological findings have been detected in the testicular tissue, which may reflect the pollution level these birds are exposed (Fig. 3).

##### *Laughing dove*

In breeding season, histopathological examination of the dove testis showed a testicular parenchymal organization of large seminiferous tubules that displayed some desquamation of germinal epithelial cells, containing sloughed secondary spermatocytes, spermatids, and spermatozoa in the tubular lumen. The seminiferous tubules exhibited also few apoptotic germ cells. The regressed testis of dove in nonbreeding season displayed more testicular injury characterized by a thick and irregular basement membrane, small lumen, and degenerated complement of germinal epithelium containing only sloughed degenerated spermatogonia, vacuolated and apoptotic spermatocytes.

##### *Barn swallows*

The testicular histological observation of swallow in breeding season revealed germinal epithelial desquamation in some seminiferous tubules, apoptosis, and degeneration in many germ cells. The regressed testis of swallow in the nonbreeding season showed involuted testicular parenchymal organization of small seminiferous tubules that displays irregular and thick basement membrane, small lumen, and degenerated

complement of germinal epithelium contained only sloughed degenerated spermatogonia, few spermatocytes, and many apoptotic and vacuolated germ cells.

##### *House sparrow*

The testicular histological examination of the sparrow in breeding season revealed germinal epithelial sloughing in some seminiferous tubules that have thick basement membranes, apoptosis, and degeneration in many germ cells. The regressed testis of the sparrow in the nonbreeding season showed involuted testicular parenchymal disorganization of small seminiferous tubules that displays irregular and thick basement membrane, small lumen and degenerated complement of germinal epithelium contained only sloughed degenerated spermatogonia, few spermatocytes, and many necrotic germ cells. Taken together, the histological changes of the testes of the house sparrow were more prominent than that in dove and swallow (Fig. 3). The regressed testicular tissue of all the males in nonbreeding season was more affected than that in the breeding season.

##### Ovary

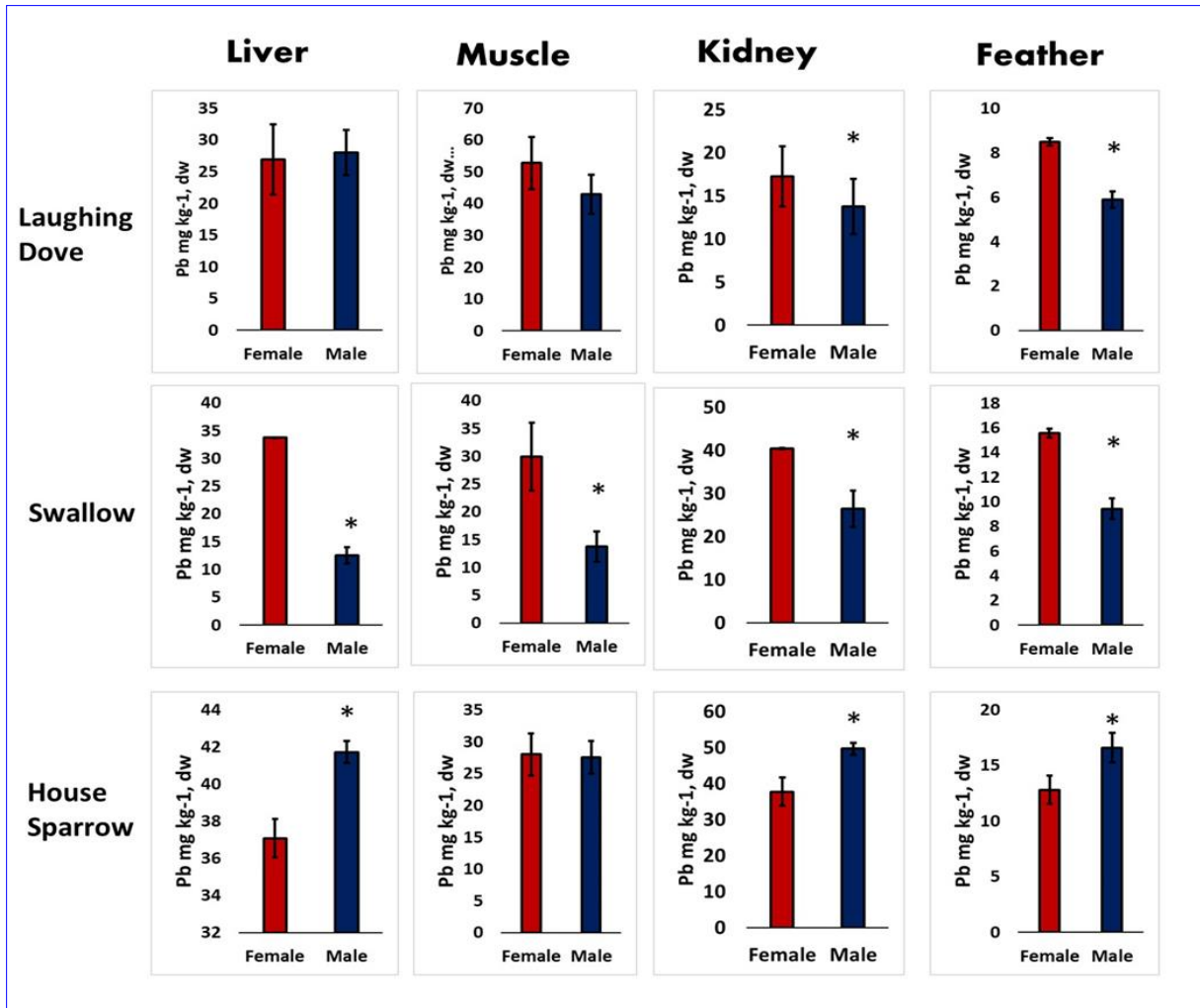
Similar to the histopathological status in testes, the ovaries of the examined wildy collected birds showed several histopathological observations, reflecting the surrounding pollution level (Fig. 4).

##### *Laughing dove*

Histologic examination of the ovary of a dove in the breeding season revealed many atretic follicles identified by their irregular shape and detached membrana granulosa. The observed regressing postovulatory follicle was characterized by the accumulation of vacuolated granulosa and theca cells. The observed ovarian cortex contained a large number of primary follicles. In nonbreeding dove, the ovary was regressed and decreased in mass and there were no mature or postovulatory ovarian follicles detected. Most premature ovarian follicles were atretic and embedded in loose ovarian stroma. Few numbers of primary follicles were observed in the ovarian cortex.

##### *Barn swallow*

Ovarian histological examination of Egyptian barnw



**Figure (1):** Pb<sup>2+</sup> concentrations in different tissues of males and females of laughing dove, swallow and house sparrow. Data are represented in mean  $\pm$  standard error of six replica for each sample of the studied species ( $n = 6$ ) in all cases. The data with asterisks (\*) are significantly different at  $p \leq 0.05$  level using unpaired t-test.

Swallow, in breeding season, revealed different stages of ovarian follicles; many of them were atretic. The ovarian tissue exhibited cortical thinning and stromal loosening. In nonbreeding season, ovarian architecture of the swallow appeared regressed with a thick ovarian cortex, a little number of the atretic follicle, and denser stroma than that in the breeding season.

#### House sparrow

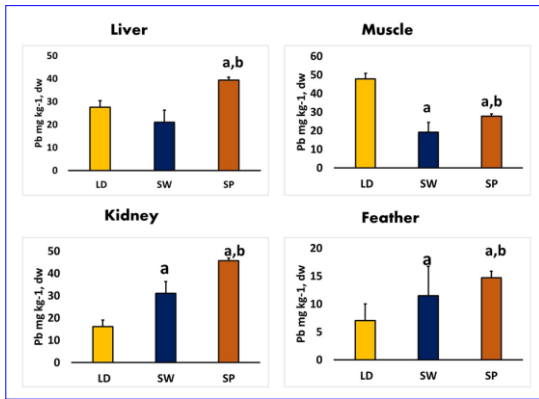
Histological examination of sparrow ovary in the breeding season shows all the different stages of ovarian follicles but most of them were atretic. The thick ovarian cortex contained a large number of primary and secondary ovarian follicles. In nonbreeding season, the regressed ovarian tissue revealed a stromal loosening and a great number of primary and secondary ovarian follicles most of them were atretic.

Collectively, the testicular tissue was generally more affected than the ovarian tissue, especially in the nonbreeding season. The ovarian architectural changes of the sparrow were more pronounced than that in the dove and swallow. These histological changes have appeared in the ovaries of the breeding and nonbreeding seasons of all the birds (Fig. 4).

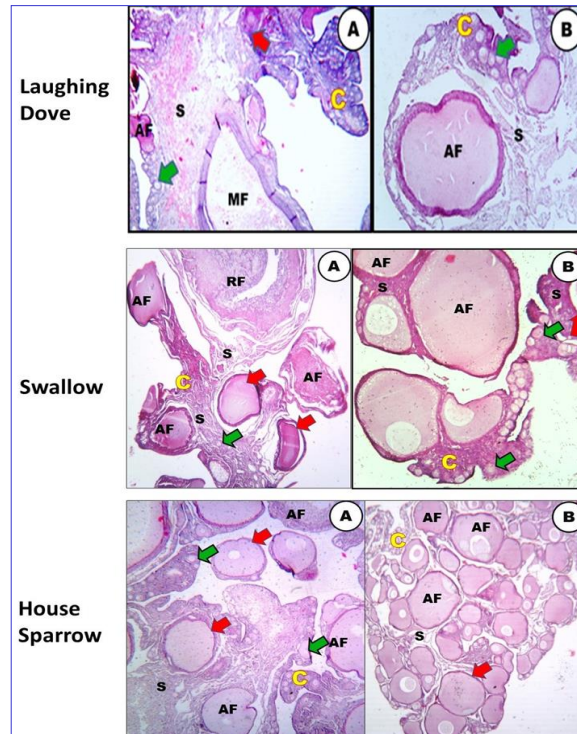
## DISCUSSION

The environmental contamination of Pb due to the human activities impact led to an accumulation of Pb in the wildlife including birds. Numerous studies have examined the accumulation of heavy metals in several avian organs, including the liver, kidney, muscle, and feathers (Chao *et al.*, 2003; Taggart *et al.*, 2006; Naccari *et al.*, 2009; Albayrak and Pekgoz, 2021). Pb had harmful consequences on birds, including effects on the neurodevelopment of chicks (Burger and Gochfeld, 1997). Most organisms normally experience a loss of body mass as a result of heavy metal buildup. For instance, (Prakash and Bhargava, 2006) discovered that Zn, Cd, and Pb decreased Kharpa beetle, *Trogoderma granarium*, larva, and pupa size.

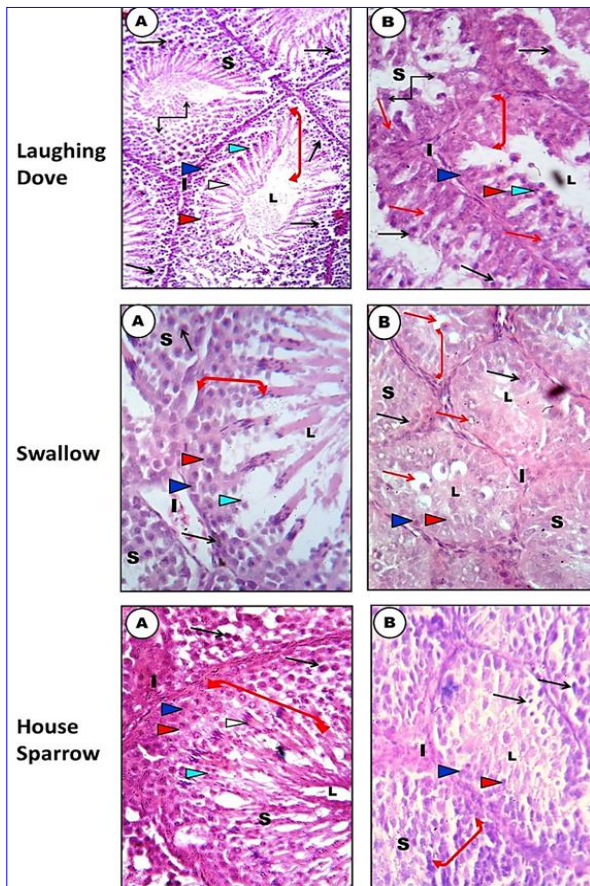
Previous research in the Damietta region indicated that soil, fish, and plants have a high concentration of Pb due to urban pollution (Mandour *et al.*, 2021; Ebeid *et al.*, 2022). In our study, the lead accumulation in different organs showed variations between males and females in the same species. The three studied birds were caught from the same habitat, which is the coastal



**Figure (2):** The Pb concentrations in liver, muscle, Kidney, and feather of laughing dove (LD), swallow (SW), and sparrow (SP). Data are presented as mean ± standard error of n =12 samples of each species. statistical difference: ANOVA p<0.05 in all subfigures; (a) denotes a significantly different value from the laughing dove value; (b) denotes a significantly different value from swallow value.



**Figure (4):** Histopathological changes observed in ovaries of wildy sampled 3 bird species from Damietta. Ovaries were collected during both breeding (A) and nonbreeding (B) seasons. The figure shows different stages of ovarian follicles. Abbreviations: AF, atretic follicle; C, cortex; MF, mature follicles; RF, regressing follicle; S, ovarian stroma or medulla; red arrow, secondary follicles; green arrow, primary follicle (H&E. X200).



**Figure (3):** Histopathological abnormalities seen in the testes of three studied bird species that collected randomly in Damietta. Testes were taken during breeding (A) and nonbreeding (B) seasons. Seminiferous tubules and intertubular regions are seen. L, tubule lumen; I, interstitial tissue; S, seminiferous tubules; dark blue arrow head, spermatogonia; red arrow head, primary spermatocyte; light blue arrow head, secondary spermatocyte; green arrow head, Sertoli cells; white arrow head, spermatids; yellow arrow head, apoptotic primary spermatocyte; yellow curved arrow, spermatozoa; red two head arrow, normal germinal epithelium; black two head arrow, degenerated germinal epithelium. (H&E. X200).

area dominated by the heavy human impact of the expansion of industries around the Damietta port. The result showed a clear distinction between different bird

species that vary in feeding habits and mating system behavior. This variability in their activity may have caused this difference in trace element pickup from the environment. Within the same species, females display more Pb accumulation than males in both laughing doves and swallows which may be explained by their foraging activity including their wide range of searching for food. Also, their mating behavior that belongs to monogamy requires equal share with males in parental care and rearing young. In contrast, house sparrows are exposed to a high dose of heavy metals, especially for males than for females. This could be explained by that house sparrows are feral birds widely distributed in all habitat types and can tolerate a wide range of destruction and pollution. It prefers to inhabit more crowded urbanized areas with high traffic, which make it exposed to Pb emission more than swallows and doves that prefer more rural areas than urbanized ones. This finding was consistent with the study done in Pakistan, they found a high concentration of Pb in house sparrows (Aziz *et al.*, 2021), especially those near heavy traffic. The consequences of pollution that could be Pb or other sources of pollutants are indicated in a clear way in the testes of house sparrow males that suffered from regressed testicular tissues more than laughing doves and swallows.

There was great variability in Pb levels among different birds. This may be illustrated by the difference in trophic position and habitat type preference for each bird that plays a determinant role in metal biomagnifications in bird tissues (Lavoie *et al.*,

2013, Samaraweera *et al.*, 2022). It was very clear from the histology of the testis and ovaries of the three studied birds that they suffer from the pollution that caused great damage to ovarian and testicular tissues.

The result showed that the testicular tissue was generally more affected than the ovarian tissue, especially in the nonbreeding season. The regressed testes of the sparrow in the nonbreeding season showed involuted testicular parenchymal disorganization of small seminiferous tubules that displayed irregular and thick basement membrane, small lumen, and degenerated complement of germinal epithelium contained only sloughed degenerated spermatogonia, few spermatocytes, and many necrotic germ cells. The ovarian architectural changes of the sparrow were more pronounced than that in the dove and swallow. These histological changes have appeared in the ovaries of the breeding and nonbreeding seasons of all the birds. This damage can lead to failure in breeding and reduce fitness. Results of the effect of Pb on these reproductive organs are in agreement with that of Williams *et al.* (2017), who reported that Pb has a wide range of adverse health impacts, including those on the liver, kidneys, immune system, and reproductive system.

### CONCLUSION

The extent of the harm documented in this study is alarming, especially when considering exposure to vehicle emissions. Additional research is needed to fully comprehend these contaminants' consequences. More species from the same foraging guild should also be included, and Pb concentrations as an abiotic scenario should be examined.

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## استخدام الطيور ذات أنظمة التزاوج وعادات التغذية المختلفة كمؤشر حيوي للتلوث بالرصاص في المناطق السكنية

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### الملخص العربي

يعتبر رصد العناصر السامة في الحياة البرية مهم جدا من اجل الصون الحيوي والحفاظ عليها. قمنا بقياس مستويات Pb في الكبد والعضلات والكلية والريش في كلا من البمام، عصفور الجنة المصري، والعصفور الدوري. وتختلف هذه الأنواع الشائعة من الطيور، واسعة الانتشار في المنطقة الساحلية من دمياط، في العادات الغذائية، وتفضيلات الموائل، وأنظمة التزاوج. تم تجميع الطيور البرية في مواسم التكاثر والراحة. كما أظهرت النتائج أن إناث البمام وعصفور الجنة كان لديها تركيزات الرصاص أعلى بكثير من الذكور. في حين أن ذكور العصفور الدوري كان تركيز الرصاص أعلى بكثير من الإناث. يمكن أن يعزى ذلك إلى البحث النشط عن الطعام في كل من إناث البمام وعصفور الجنة، وذكور العصافير. كما أظهرت النتائج أن سلوك البحث عن الطعام وتفضيل الموائل أثر على تركيزات الرصاص. أظهرت العصافير الدورية واسعة الانتشار بالقرب من الطرق الرئيسية تركيزا أعلى من الرصاص، خاصة في الكبد والكلية والريش من البمام وعصفور الجنة، والتي تفضل المناطق الريفية البرية. كشف الفحص النسيجي للغدد التناسلية عن خصيتين ومبايض غير صحية. كان نسيج المبيض أقل تأثرا من أنسجة الخصية، خاصة خلال موسم الراحة. بالمقارنة مع البمام وعصفور الجنة، كانت النتائج النسيجية المرضية أكثر وضوحا. يبدو أن الخصيتين كانتا أكثر تأثرا وأكثر ارتباطا بنتائج قياسات الرصاص، ويمكن استخدامها كمؤشر حيوي لتلوث المعادن الثقيلة. تقدم النتائج أيضا بعض الأدلة على إمكانات العصفور الدوري كمراقب حيوي للتلوث بالمعادن الثقيلة في المناطق الحضرية. يؤكد هذا البحث على أهمية استخدام الدلائل الحيوية من مختلف أنواع الموائل والتغذية المختلفة. من أجل إنشاء برنامج طويل الأجل للرصد البيولوجي لحفظ الطيور في كل من السياق المحلي والعالمية، ومن الضروري إنشاء بيانات عن تعرض الحياة البرية للعديد من العناصر الضارة.