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Impact of urban lead pollution on the hematology and reproductive histopathology of stray cats in Indonesian megacities

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Abstract Lead pollution in urban environments has become a significant concern owing to its potential impact on the health of wildlife, including cats. This study aimed to evaluate the effects of lead exposure on the hematological parameters and reproductive histopathology of stray cats in Jakarta and Surabaya. The toxicity of inhaled lead contamination is distributed throughout the body via the bloodstream, affecting the reproductive system through hormonal disruption by reducing hypothalamic function and through cytotoxicity by inducing reactive oxygen species. Bioindicators in the form of male wild cats were used as experimental animals and were selected through accidental sampling over a 24-h period, with at least five animals collected from each region. Blood samples were utilized for hematological analysis and blood lead concentration measurement, whereas testicular tissues were prepared for histopathological examination. Although the serum lead levels of the cats were within the safe limits set by the World Health Organization, several morphological abnormalities in the blood cells were noted, including basophilic stippling and the presence of clover-leaf cells, indicating the toxic effects of lead exposure. Additionally, histopathological analysis revealed seminiferous tubule degeneration and atrophy, which could affect reproductive function. These findings suggest that although lead exposure remains within tolerable limits, its implications for the reproductive health of stray cats warrant further attention. This study highlights the importance of monitoring lead pollution in urban areas and underscores the need for conservation measures to protect stray cat populations and their ecosystems.

Keywords: air pollution, lead toxicity, urban cats, reproductive health, environmental biomarkers

1. Introduction

Environmental pollution refers to the dispersal of certain chemical elements that can disrupt the environmental balance (Lead et al., 2018). Rapid industrial development has been accompanied by increasing environmental pollution owing to industrial waste when industries do not properly manage and treat waste (Osman 2014). Urban areas, which are densely populated, experience air pollution from vehicle emissions, industrial gases, power plant emissions, cigarette smoke, and other human activities (Goodsite et al., 2021). In Indonesia, approximately 70% of air pollution is caused by motor vehicle emissions containing lead (Pb), suspended particulate matter, nitrogen oxides, hydrocarbons, carbon monoxide, and photochemical oxidants (Surya et al., 2020).

Despite significant regulatory efforts, air pollution continues to pose a serious threat to public health and urban ecosystems, particularly in densely populated cities, such as Surabaya and Jakarta (Soemarko et al., 2023). However, research on the specific impacts of Pb contamination on urban wildlife, particularly stray animals, remains limited. In Indonesia, air, water, and soil pollution in urban areas, particularly from Pb contamination, has reached alarming levels. In 2016, the air Pb concentration in Surabaya exceeded the US Environmental Protection Agency standard of 0.15 µg/Nm3, reaching 0.30 µg m^{-3} (Lestiani et al., 2024). Similarly, another study indicated that approximately 12% of Jakarta's area had Pb concentrations exceeding the threshold (Siregar et al., 2016). Emissions from motor vehicles, industrial activities, and other urban sources primarily contribute to these elevated Pb levels (Liu et al., 2014).

Pb is a pervasive environmental contaminant, and its bioaccumulation in various species can cause severe health problems, particularly affecting organ systems responsible for reproduction, blood production, and detoxification (Ali et al., 2019; Singh et al., 2023; Aljohani, 2023). Chronic exposure to low Pb doses remains an underestimated hazard in developing countries, where urban infrastructure

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struggles to cope with industrial growth (Stewart and Hursthouse, 2018). When inhaled, Pb is absorbed into the bloodstream and distributed throughout the body, affecting various tissues and organs (Assi et al., 2016). Chronic Pb exposure disrupts heme biosynthesis, thereby leading to anemia characterized by a reduced erythrocyte count and hemoglobin quality (Ugwuja et al., 2020). Pb-exposed erythrocytes may develop basophilic stippling, a condition in which blue–black granules appear in erythrocytes due to ribosomal RNA condensation or precipitation resulting from hemoglobin synthesis abnormalities (Zachary, 2017).

In addition to blood toxicity, Pb exposure has profound effects on reproductive systems, as demonstrated by several studies across different species (Foster, 1992; Eldridge and Stevens, 2016; Anyanwu and Orisakwe, 2020). Although studies have examined the general effects of Pb exposure on the blood and reproductive systems of laboratory animals, fewer studies have focused on stray animals in urban settings, where chronic exposure to low levels of environmental pollutants is more prevalent. This disruption is concerning, particularly in urban fauna, which experience prolonged exposure to environmental pollutants. In particular, the effects of Pb on the reproductive health of stray male cats, which are bioindicators of urban pollution, have not been extensively investigated. To develop effective public health interventions, understanding the reproductive and hematological impacts of Pb toxicity is essential (Mitra et al., 2017). Various organ systems, including the blood, nervous system, kidneys, reproductive system, and digestive system, are affected by Pb accumulation (Collin et al., 2022). Elevated blood Pb levels are associated with an increased risk of hypertension, kidney disease, reproductive dysfunction, and other health issues (Kumar, 2018). Reproductive system disruptions include infertility, increased miscarriages, stillbirths, premature births, and reduced sexual desire. Environmental exposure to cadmium, Pb, and mercury has shown reproductive organ toxicity (Massányi et al., 2020). Additionally, Pb-induced oxidative stress increases the level of reactive oxygen species, thereby leading to significant damage to the reproductive system (Kelainy et al., 2019; Prastiya et al., 2023). Considering the severe implications of Pb exposure, identifying effective bioindicators that can monitor environmental Pb pollution and its biological effects is critical (Zukal et al., 2015). Owing to their high exposure to urban pollution, male stray cats constitute a promising model for investigating the hematological and reproductive impacts of Pb toxicity.

The use of biomarkers as indicators of environmental Pb exposure offers a viable approach for assessing toxicity risks in urban animal populations. Stray animals, including cats, provide insight into chronic low-dose exposure levels in environments heavily affected by industrial pollution. Hematological and histopathological changes are particularly direct indicators of Pb toxicity and its biological effects on exposed organisms. Characteristic abnormalities in tissues and cells can act as biomarkers for environmental quality assessment against specific toxic effects. Biomarkers, which are signaling indicators in biological systems, measure environmental quality via bioindicators (Aliyu Haruna Sani and Umar Sidi Jamilu, 2022). Biomarkers are crucial for the early detection of Pb pollution impacts, enabling preventive measures and ecosystem recovery (Santoso et al., 2022). This study used blood and reproductive organ samples from stray male cats as bioindicators for Pb exposure owing to their direct inhalation exposure in industrial areas.

This study aimed to investigate the hematological and histopathological impacts of Pb exposure on the reproductive organs of male stray cats in the megacities of Jakarta and Surabaya. By analyzing blood and testicular samples from these cats, we sought to measure their serum Pb levels to assess the extent of environmental contamination; analyze changes in hematological parameters, including erythrocyte count, hemoglobin quality, and the presence of basophilic stippling; identify signs of Pb-induced anemia and other blood disorders; and perform histopathological examinations of testicular tissues to observe any structural and cellular changes indicative of Pb toxicity, including oxidative stress, tissue necrosis, and disrupted spermatogenesis. In addition, we aimed to confirm the suitability of male stray cats as bioindicators for monitoring low-dose Pb exposure in urban environments, thereby providing valuable data for environmental health assessments and public health interventions. By achieving these objectives, this study contributes to a better understanding of the environmental and biological impacts of Pb pollution in urban areas, supporting efforts to mitigate pollution and protect animal and human health.

2. Methodology

2.1. Study design and location

This study was conducted to evaluate the hematological and histopathological impacts of Pb exposure on the reproductive organs of male stray cats in the megacities of Jakarta and Surabaya. This study was conducted from July to November 2024. Blood and testicular samples were collected from stray cats in the following two clinics: Cafeteriner Clinic, Jl. Raya Mastrip No. 26, Kedurus, Karangpilang, Surabaya, East Java and Yayasan Peduli Lingkungan Indonesia Clinic, Jl. Tanah Merdeka No. 54, RT.9/RW.5, Rambutan, Ciracas, East Jakarta, Special Capital Region of Jakarta.

The histopathological preparation of testicular tissues was performed at the Department of Pathology, Faculty of Veterinary Medicine, Universitas Airlangga. The Pb concentration of the blood samples was measured at the Balai Besar Laboratorium Kesehatan Jakarta. Blood smear analysis and histopathological observations were conducted at Integrated Laboratory 1, Faculty of Health Sciences, Medicine, and Life Sciences, Universitas Airlangga, Jalan Wijayakusuma No. 113, Banyuwangi.

2.2. Animal selection and sampling

Using accidental sampling within a 24-h time frame, stray male cats were selected, ensuring a minimum of five cats from each city (Jakarta and Surabaya). The selection criteria were based on visual observation, with a focus on adult male cats without overt signs of illness or injury. Stray cats were captured at locations with known high pollution levels, such as near busy roads and industrial areas, to ensure representative sampling. Preoperatively, the stray cats were fasted for 8 h to minimize the risk of anesthesia-induced complications. The anesthetic protocols involved a combination of xylazine and ketamine hydrochloride. To achieve adequate sedation and analgesia for the procedure, each cat received an intramuscular injection of xylazine (1–2 mg/kg), followed by ketamine hydrochloride (5–10 mg/kg).

The castration procedure began with the cats being placed in dorsal recumbency, lying on their back, and the surgical area was shaved and disinfected with a povidone–iodine solution (Betadine®, Mundipharma Healthcare, Jakarta, Indonesia). To expose the testicles, a midline scrotal incision was created. Each testicle was subsequently exteriorized, and the spermatic cord was ligated via an absorbable catgut suture (Safil®, B. Braun, Melsungen, Germany). The testicle was excised distal to the ligature, and the scrotal incision was left open to heal by second intention, a common practice in cat castrations for reducing the risk of postoperative complications.

Using a 3-mL syringe, blood samples were collected from the cephalic vein. The area over the cephalic vein was clipped and disinfected, and a tourniquet was applied proximal to the site of venipuncture to engorge the vein. Approximately 3 mL of blood was drawn and divided into two aliquots: one for Pb concentration analysis and the other for hematological examination. To prevent coagulation, the blood for Pb analysis was placed into heparinized tubes.

To remove any blood or contaminants, the excised testes were immediately washed with sterile physiological saline. To preserve the tissue architecture, the testes were subsequently fixed in 10% neutral buffered formalin for at least 24 h. Following fixation, the tissues were dehydrated through a series of graded alcohols, cleared in xylene, and embedded in paraffin wax. The paraffin blocks were sectioned at a thickness of 5 µm via a microtome, and the tissue sections were mounted on glass slides and stained with hematoxylin and eosin (H&E) for general histopathological examination. This comprehensive procedure ensured the collection of high-quality samples for subsequent analyses of the Pb concentration, hematological changes, and histopathological effects.

2.3. Pb concentration measurement

To determine Pb concentrations, blood samples collected from stray male cats were analyzed via atomic absorption spectrophotometry (AAS), the gold-standard technique for trace metal analysis. To prevent coagulation and hemolysis, the samples were stored in heparinized tubes. To prepare the blood samples for AAS analysis, a digestion process was performed to breakdown the organic matrix and release the metal ions into a measurable form. Each blood sample (0.5 mL) was transferred into a digestion flask containing a concentrated nitric acid and perchloric acid mixture at a 4:1 ratio. The flask was placed on a hot plate under a fume hood; to facilitate the digestion process, the temperature was gradually increased to 150°C. The samples were heated until a clear solution was obtained, indicating complete digestion of the organic material. After cooling, the digested samples were diluted with deionized water to a final volume of 10 mL and filtered through a 0.45-µm membrane filter to remove any particulate matter.

The Pb concentrations in the prepared samples were measured via a PerkinElmer AAnalyst™ 800 atomic absorption spectrophotometer equipped with a graphite furnace to increase the sensitivity and accuracy of low-level Pb detection. To generate a calibration curve, calibration standards were prepared from a certified Pb standard solution with concentrations of 0–50 µg/dL. Each sample was aspirated into a graphite furnace, and the absorbance was measured at a 283.3-nm wavelength, which is specific for Pb detection.

The quality control procedures included the use of blank samples, spiked samples, and certified reference materials to ensure measurement accuracy and precision. Blank samples were run to account for any potential contamination or background interference, whereas spiked samples (blood samples with known Pb additions) were analyzed to verify the recovery rates of the digestion and measurement processes. To validate the method’s reliability, certified reference materials with known Pb concentrations were analyzed alongside the samples. The Pb concentrations in the blood samples were calculated on the basis of the absorbance readings and the calibration curve. The results are expressed in micrograms per deciliter (µg/dL). The data provide critical information on the extent of Pb exposure in stray male cats from Jakarta and Surabaya, offering insights into the environmental contamination levels in these urban areas.

2.4. Hematological analysis

To assess the impact of Pb exposure on the blood parameters of stray male cats from Jakarta and Surabaya, hematological analysis was performed. Blood samples collected in EDTA tubes were used for this purpose. The preparation of blood smears was the first step in hematological analysis. A drop of blood was placed on a clean glass slide, and another slide was used to spread the blood into a thin layer. The prepared blood smear was air dried and subsequently fixed in methanol for 5 min to preserve the cell morphology. The fixed smears were stained via Wright–Giemsa stain, a combination of eosin and methylene blue, which differentially stains cellular components to increase visibility under a microscope.

To identify and quantify erythrocyte morphology and abnormalities, the stained blood smears were examined under a light microscope at 1,000× magnification via oil immersion. The interpretation of the hematological results focused on correlating the observed abnormalities with the known effects of Pb toxicity.

2.5. Histopathological examination

Testicular tissues were fixed in formalin, embedded in paraffin, sectioned at 5 µm, and stained with H&E. Identifying structural and cellular changes, including oxidative stress markers, tissue necrosis, and disruptions in spermatogenesis, was the focus of histopathological analysis. Leydig and spermatogenic cells were specifically evaluated for pathological alterations.

2.6. Data analysis

To summarize the Pb concentrations, hematological parameters, and histopathological findings, the data were analyzed via descriptive statistics. To assess the significant differences and correlations between Pb exposure and observed health effects, comparative analysis between samples from Jakarta and Surabaya was performed via appropriate statistical tests.

3. Results

3.1. Blood smear observations

Hematological analysis of blood smears from stray male cats in Jakarta and Surabaya revealed several abnormalities due to Pb exposure (Figure 1). Significant morphological changes were observed in the blood cells despite Pb levels being within tolerable limits for cats as bioindicators (Figure 3). Blood smears revealed basophilic stippling in erythrocytes, characterized by small dark blue granules, which are a hallmark of Pb toxicity. Moreover, clover-leaf cells were detected in relatively low numbers; these abnormally shaped erythrocytes are specific indicators of Pb exposure. Other observed abnormalities included microcytosis and macrocytosis, with the presence of both abnormally small and large red blood cells, teardrop cells, and Burr cells. Further irregularities, including teardrop cells, Burr cells, and stomatocytes, were also noted, reflecting disrupted erythropoiesis and cell morphology. These hematological findings underscore the impact of Pb exposure on the erythrocyte population, suggesting Pb-induced anemia and cellular damage.

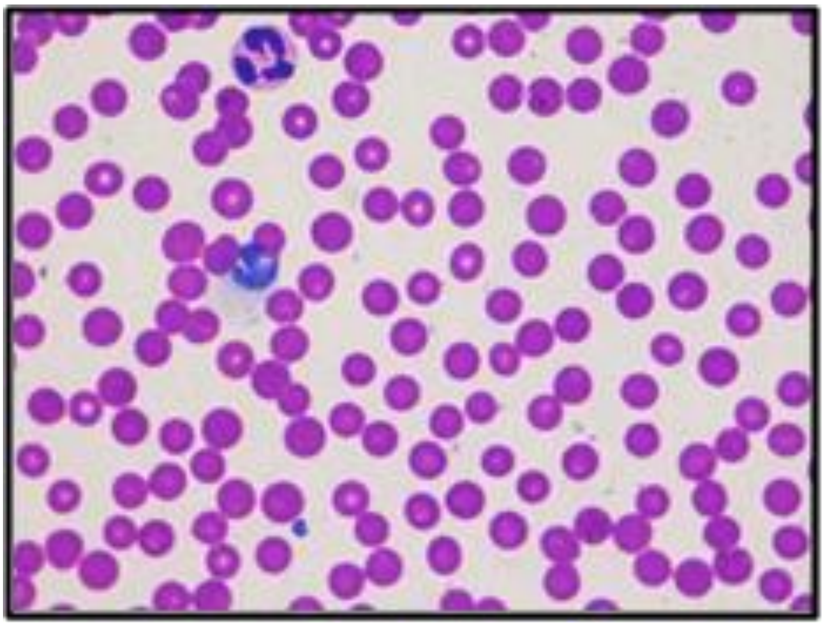

Figure 1 Representative image of a normal blood smear observed under a trinocular microscope at 100× magnification.

3.2. Pb concentration in blood

The Pb concentration in the blood was measured via AAS at 283.53 nm. The results revealed that the Pb levels in the blood samples were within the permissible limits set by the World Health Organization (WHO), with a maximum allowable concentration of 10 mg/dL. Detailed data on the Pb concentrations in the blood samples from Jakarta and Surabaya are presented in Tables 1 and 2, respectively, showing ranges of 1.0–1.6 and 1.0–1.5 mg/dL in Jakarta and Surabaya, respectively. These levels, although below the WHO threshold, indicate measurable exposure to Pb in the stray cat population from both cities.

Table 1 Blood lead (Pb) levels in the cat samples from Jakarta and Surabaya.

No.	Samples	Pb Level (mg/dL)	Samples	Pb Level (mg/dL)
1.	Male Stray Cat 1, Jakarta	1.6	Male Stray Cat 1, Surabaya	1.3
2.	Male Stray Cat 2, Jakarta	1.0	Male Stray Cat 2, Surabaya	1.2
3.	Male Stray Cat 3, Jakarta	1.5	Male Stray Cat 3, Surabaya	1.5
4.	Male Stray Cat 4, Jakarta	1.5	Male Stray Cat 4, Surabaya	1.5
5.	Male Stray Cat 5, Jakarta	1.4	Male Stray Cat 5, Surabaya	1.4
6.	Male Stray Cat 6, Jakarta	1.1	Male Stray Cat 6, Surabaya	1.0
7.	Male Stray Cat 7, Jakarta	1.3	-	
8.	Male Stray Cat 8, Jakarta	1.2	-	

Among the samples from cats in Jakarta, the highest observed Pb concentration was 1.6 mg/dL, whereas the lowest was 1.0 mg/dL. The mean concentration suggested that Pb exposure was present but remained within a range that could be metabolically managed through natural excretion processes, such as urination and defecation. Similarly, in the sampled cats from Surabaya, the highest observed Pb concentration was 1.5 mg/dL, with the lowest being 1.0 mg/dL (Table 1). These levels indicate Pb exposure but within a range that does not exceed the body's capacity for detoxification, suggesting that although the stray cats were exposed to Pb, the concentrations were low enough to be processed without causing severe toxicity.

3.3. Histopathological examination of the testicular tissues

Histopathological analysis of the testicular tissues from the sampled cats revealed varying degrees of abnormalities consistent with Pb exposure. In several samples, the seminiferous tubules appeared normal, with a dense population of spermatogenic cells and abundant spermatozoa in the lumen (Figure 2). However, some samples displayed marked abnormalities. Spermatogenic cell degeneration was indicated by vacuolization within the seminiferous tubules. Seminiferous tubule atrophy is characterized by a reduced diameter and thickening of the germinal epithelium, with some tubules containing only Sertoli cells. Additionally, disorganized spermatogenic cells with structural irregularities and a reduced number of spermatogenic cells were observed, suggesting disrupted spermatogenesis. These findings indicate that Pb exposure, even at low levels, can cause significant morphological changes in reproductive organs, potentially affecting fertility.

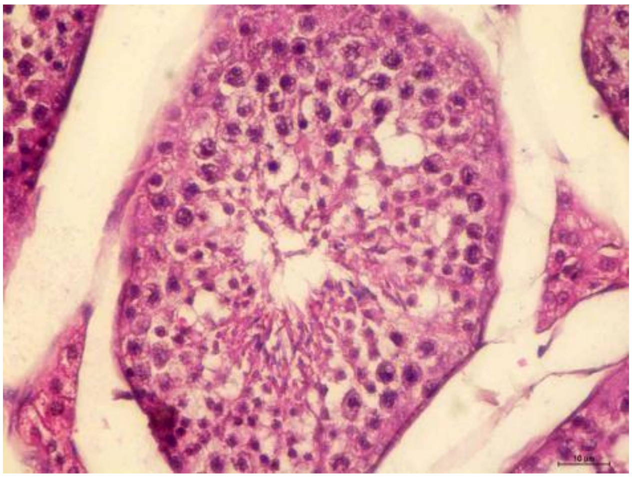

Figure 2 Histological image of normal seminiferous tubules observed under a trypsin microscope at 40× magnification.

Overall, the examination of the testicular preparations revealed a generally normal condition characterized by a dense structure and a sufficient number of spermatogenic cells. Additionally, the lumens of the seminiferous tubules contained many spermatozoa. The presence of Leydig cells under normal conditions and in significant numbers play crucial roles in the spermatogenesis cycle, contributing to the production of normal spermatozoa. However, several preparations also exhibited histopathological abnormalities, as illustrated in the following images:

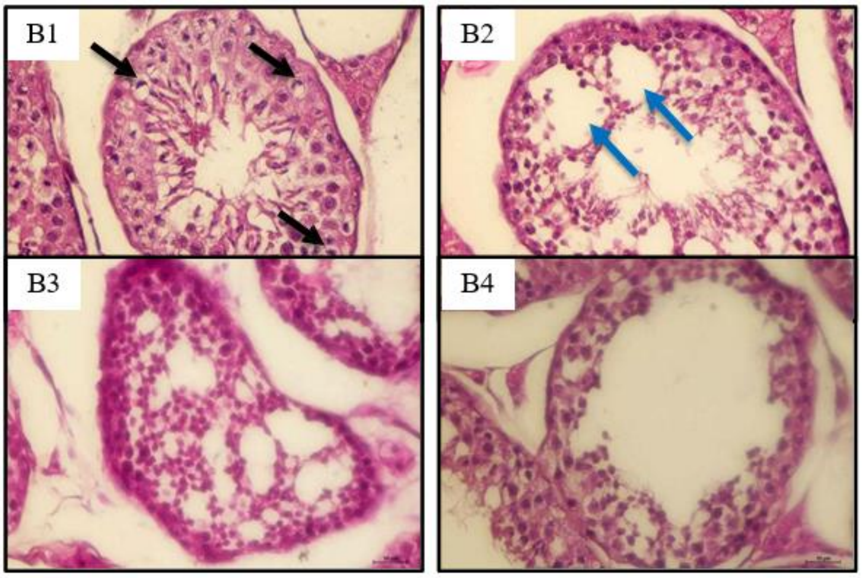

Figure 3 Histopathological image of the seminiferous tubules. The observed abnormalities included spermatogenic cell degeneration (B1), seminiferous tubule vacuolization (B2), disorganized spermatogenic cell structure in the seminiferous tubules (B3), and seminiferous tubule atrophy and lumen diameter enlargement (B4). The samples were observed under a trypsin microscope at 40× magnification.

The progressive degeneration and phagocytosis of spermatogenic cells lead to lesions, including seminiferous tubule atrophy, which is characterized by the absence of spermatogenic cells within the tubules, thereby resulting in the tubules being composed solely of Sertoli cells. The seminiferous tubule atrophy and decreased number of spermatogenic cells are morphological indicators of spermatogenesis cycle failure. The spermatogenesis cycle can be negatively affected by a decreased number of spermatogenic cells, thereby leading to a reduced seminiferous tubule diameter.

3.4. Results of the histopathological observations of the epididymis

Histopathological preparations of the epididymis revealed that several samples presented abnormalities, including empty lumens in the epididymis. This appearance of empty lumens indicates decreased spermatozoa production in the testes. Various factors may cause these abnormalities, one of which is exposure to the toxic effects of heavy metals, including Pb. Below are images illustrating the histopathological findings of the epididymis (Figure 4):

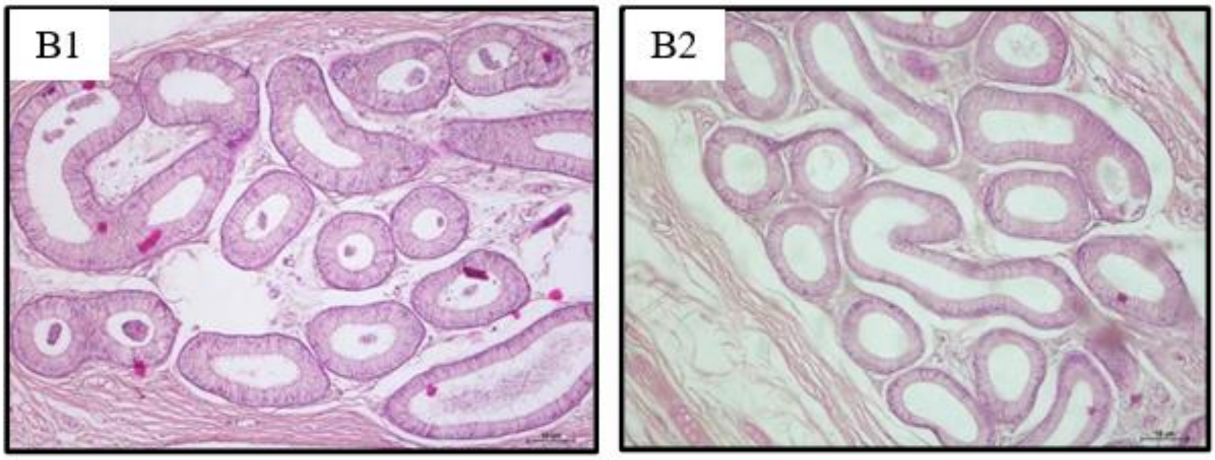

Figure 4 Histopathological image of the epididymal tubules. The lumen of the epididymal tubules is filled with spermatozoa (B1); the lumen of the epididymal tubules appears empty, with no spermatozoa present (B2). The samples were observed under a trypsin microscope at 10× magnification.

The empty lumen of the epididymal tubules represents a pathological condition that indicates a failure of the spermatogenesis cycle to produce spermatozoa. The epididymis is a long duct that plays a crucial role in fluid absorption from the seminiferous tubules of the testes and the maturation, storage, and transport of spermatozoa to the ductus deferens before they join the seminal plasma and ejaculate into the female reproductive tract (Table 2).

Table 2 Histopathological observations of the samples from the testes and epididymis.

No.	Sample	Leydig Cells	Spermatogenic Cells	Epididymis
1.	Male Stray Cat 1, Jakarta	Normal	Seminiferous tubule vacuolization and disorganized spermatogenic cell structure in the seminiferous tubules	Normal
2.	Male Stray Cat 2, Jakarta	Normal	Normal	Normal
3.	Male Stray Cat 3, Jakarta	Normal	Seminiferous tubule vacuolization and spermatogenic cell degeneration	Seminiferous tubule lumen appears empty
4.	Male Stray Cat 4, Jakarta	Normal	Seminiferous tubule vacuolization	Normal
5.	Male Stray Cat 5, Jakarta	Normal	Seminiferous tubule vacuolization and disorganized spermatogenic cell structure in the seminiferous tubules	Normal
6.	Male Stray Cat 6, Jakarta	Normal	Seminiferous tubule vacuolization and disorganized spermatogenic cell structure in the seminiferous tubules	Seminiferous tubule lumen appears empty
7.	Male Stray Cat 7, Jakarta	Normal	Disorganized spermatogenic cell structure, seminiferous tubule atrophy, and enlarged lumen	Seminiferous tubule lumen appears empty
8.	Male Stray Cat 8, Jakarta	Normal	Normal	Normal
9.	Male Stray Cat 1, Surabaya	Normal	Spermatogenic cell degeneration and seminiferous tubule vacuolization	Normal
10.	Male Stray Cat 2, Surabaya	Normal	Normal	Normal
11.	Male Stray Cat 3, Surabaya	Normal	Seminiferous tubule vacuolization and disorganized spermatogenic cell structure in the seminiferous tubules	Normal
12.	Male Stray Cat 4, Surabaya	Normal	Seminiferous tubule vacuolization	Normal
13.	Male Stray Cat 5, Surabaya	Normal	Seminiferous tubule vacuolization, spermatogenic cell degeneration, and disorganized spermatogenic cell structure in the seminiferous tubules	Seminiferous tubule lumen appears empty
14.	Male Stray Cat 6, Surabaya	Normal	Disorganized spermatogenic cell structure and spermatogenic cell degeneration	Normal

4. Discussion

The inhalation contamination of the heavy metal Pb can originate from various industries that use Pb as a raw material (Naja and Volesky, 2017). The sampling of stray cats in Surabaya was conducted around the Wiyung market, which is close to the pipe and ceramic industries, whereas the sampling of stray cats in Jakarta occurred around machinery industries, industrial gas suppliers, and industrial equipment suppliers. The activities of these industries contribute to potentially high Pb contamination levels in the air, which is reinforced by data reporting Pb concentrations in the air in Surabaya in 2022, which exceeded the standard set by the US EPA at 4.2 µg m^{-3} (Santoso et al., 2022).

Monitoring data on air quality from the Jakarta Environmental Agency in 2023 revealed the highest air pollutant levels in Central Jakarta, with PM2.5 levels reaching >112 µg m^{-3} (Istiana et al., 2023). This location is not far from the sampling area around TMII. Additionally, measurements of the ambient air quality in Surabaya indicated that its ambient air quality was classified as moderate; however, on the basis of National Ambient Air Quality Standards calculations, the air quality in Surabaya was categorized as unhealthy for the community (Anggraini et al., 2024). The data obtained from Surabaya and Jakarta align with the research timeline and validate the potential high levels of airborne Pb contamination.

The results of this study indicate that although the serum Pb levels are below the WHO threshold, the observed hematological abnormalities suggest a significant impact on the physiological functions of stray cats. The presence of basophilic stippling in erythrocytes strongly indicates chronic Pb exposure, which clearly disrupts the normal erythropoiesis process (Ray, 2016). This finding aligns with similar studies on other urban animal populations, such as bats, which have exhibited hematological changes, including anemia, associated with lead exposure (Zukal et al., 2015). Comparative analysis with these taxa may provide broader insights into urban pollution impacts. Furthermore, changes in the size and shape of red blood cells, including microcytosis, macrocytosis, and teardrop cells, reflect the toxic effects of heavy metals on cellular morphology (Owonikoko et al., 2022). At the reproductive level, testicular histopathology reveals structural damage that can potentially disrupt spermatogenesis, including spermatogenic cell degeneration and disorganization as well as seminiferous tubule atrophy (Alkandurur and Kum, 2021). This finding is consistent with reports on the toxic effects of heavy metals on the reproductive functions of animals, reinforcing the hypothesis that Pb exposure, even at subchronic levels, can interfere with fertility in stray cats. Beyond physical health, lead exposure may also alter behavior and ecology. Chronic exposure in mammals is linked to behavioral changes, such as reduced exploration and cognitive impairment, potentially affecting stray cats' ability to forage, avoid predators, or interact socially in urban settings.

The results of the serum Pb concentration tests revealed that the highest concentration recorded in Jakarta reached 1.6 mg/dL, whereas the lowest was 1.0 mg/dL. In Surabaya, the highest concentration also reached 1.5 mg/dL, with the lowest at 1.0 mg/dL. The results indicate that Pb contamination can be classified as exposure, albeit at low levels that are not considered hazardous to health; therefore, the sampled cats required no preventive medical interventions. This low Pb concentration occurs because not all Pb that enters the body accumulates; metabolism plays a crucial role in the excretion of this toxic substance. Pb is excreted through feces and sweat, aided by chelating agents (Gerhardsson and Kazantzis, 2015). Chelating agents are compounds that can bind with other metals, including Pb, owing to their metal ions and chelating agents. Chelating agents that bind to Pb function as antioxidants, enabling Pb to be excreted via feces, urine, and sweat (Gulcin and Alwasel, 2022). Some of the Pb that enters the body is excreted through feces and sweat, whereas the remainder is dissolved in the blood after several hours (Grant, 2020).

Although the finding of serum Pb levels not exceeding the normal threshold was correlated with the observations of the blood smear and histopathological findings of the testes, which were mostly normal, certain pathological conditions were observed. These pathological conditions include the presence of clover-leaf cells and basophilic stippling in the blood smear. Other abnormalities noted in the blood smear include microcytic cells, which have a diameter of <6 µm and are typically caused by iron (Fe) deficiency anemia. Macrocytic cells have diameters ranging from 9--12 µm and generally present with vitamin B-12 and folate deficiency anemia. Microcytic and macrocytic cells are considered normal if they do not exceed 10%. Elliptocytes, which are oval-shaped erythrocytes, demonstrate increased osmotic fragility. Elliptocytes develop owing to cholesterol accumulation in the membrane. Burr cells are characterized by blunt regular cytoplasmic projections caused by fibrin tissue disruption. Schistocytes are irregularly shaped erythrocytes resulting from fragmentation released into the circulation by the reticuloendothelial system. Stomatocytes exhibit pale elongated erythrocytes in the center due to increased sodium and decreased potassium levels in the cells. Normal stomatocyte levels should not exceed 5%. Teardrop cells are teardop-shaped erythrocytes present in anemia due to folate and vitamin B-12 deficiencies.

Heavy metals, including Pb, can negatively impact the circulatory system, as evidenced by several morphological changes (Collin et al., 2022). The presence of clover-leaf white blood cells (leukocytes) and basophilic stippling in red blood cells (erythrocytes) are examples of such changes. Basophilic stippling is characterized by the accumulation of small basophilic granules within erythrocytes and is a reliable indicator of Pb exposure (Ray, 2016). Blood smear observations revealed various erythrocyte abnormalities, including changes in size, shape, color, and the presence of inclusion bodies. The size abnormalities included macrocytosis (enlarged erythrocytes) and microcytosis (reduced erythrocytes). The observed abnormalities in shape included burr cells (erythrocytes with blunt cytoplasmic projections), elliptocytes (oval-shaped erythrocytes), stomatocytes (erythrocytes with an elongated pale center), schizocytes (irregularly shaped erythrocytes), and teardrop cells (teardop-shaped erythrocytes). These morphological changes indicate a significant disruption in erythropoiesis due to Pb exposure, further corroborating the toxic effects of Pb on the hematological parameters of the sampled stray cats.

Iron deficiency anemia results from insufficient Fe levels in the blood, hindering erythrocyte formation and consequently leading to low hemoglobin levels. Fe plays a critical role in the formation of heme, which binds with globin chains to subsequently form hemoglobin, which is essential for erythrocyte production (Hoenemann et al., 2021). Fe deficiency can arise from various factors, including bleeding, increased physiological needs, inadequate Fe absorption, and intervention in heavy metal toxicity (Shubham et al., 2020). Pb, as a heavy metal, inhibits the binding of Fe to protoporphyrin IX in hemoglobin formation by hindering the activity of the enzyme aminolevulinic acid dehydratase (ALAD). ALAD inhibition disrupts the conversion of aminolevulinic acid (ALA) to porphobilinogen, thereby leading to ALA accumulation in the plasma. The binding of Pb to the ALAD group results in the formation of an intermediate porphobilinogen, halting the process and thereby preventing hemoglobin formation (Wang et al., 2010).

Histopathological observations of the samples from the testes and epididymis of the cats revealed that the majority of the samples appeared normal. This finding is correlated with blood Pb levels, which are categorized as low exposure and do not exceed the normal threshold. The Pb levels remained within the normal range, indicating that there was no negative impact on the testes or epididymis, as evidenced by the predominantly normal structure of these organs on histopathological examination.

However, some abnormalities, including spermatogenic cell degeneration, seminiferous tubule vacuolization, irregular structure of the spermatogenic cells within the seminiferous tubules, seminiferous tubule atrophy, and lumen diameter enlargement, were observed. Considering that the blood Pb levels remain categorized as exposed but not hazardous, these observed abnormalities are not attributed to Pb exposure. The abnormalities likely arise from other factors contributing to the observed histopathological findings. These other factors may include exposure to other heavy metals, such as cadmium, which can also have toxic effects on the reproductive system.

This study contributes to a deeper understanding of the impact of exposure to heavy metals, particularly Pb, on the hematological health and reproductive function of wildlife in urban environments. Although blood Pb levels are within safe limits according to WHO standards, hematological and histopathological findings indicate that long-term Pb exposure can cause significant damage (Debnath et al., 2019). This study reinforces the importance of considering the impact of urban environments on animal health, especially for species frequently exposed to contaminants, such as Pb. In practical terms, these results encourage the implementation of pollution monitoring and control programs in urban areas to protect urban animal populations.

This study had several limitations. The primary limitation of this study was the lack of longitudinal data that could be used to evaluate the long-term effects of Pb exposure on reproductive and hematological health. The cross-sectional nature of this study provides only a snapshot of the condition of stray cats, making determining whether the observed effects would worsen over time difficult. Furthermore, other environmental factors that may play a role in hematological and reproductive changes, such as parasitic infections or malnutrition, which could also affect outcomes, were not addressed in this study.

These findings have significant social implications for the health of urban wildlife, which are frequently exposed to various pollutants, including Pb. Although the blood Pb levels in stray cats remain below the WHO threshold, their impact on health cannot be overlooked. From an ethical perspective, this study highlights the urgent need to control Pb pollution in urban areas, not only to protect wildlife but also to protect human populations living in the same environment. Although lead exposure in stray cats did not exceed hazardous levels, it serves as a bioindicator for urban environmental health. The findings underscore the relevance to public health, as the presence of lead in stray animals reflects shared environmental contamination that could affect human populations. Addressing urban lead pollution can benefit both wildlife and human communities.

5. Conclusion

Here, we identified the significant impacts of Pb exposure on the hematological health and reproductive histopathology of stray cats in Jakarta and Surabaya. Although the blood Pb levels are within acceptable limits according to the WHO, hematological analyses revealed various abnormalities that indicate a tangible health risk. The observed abnormalities, including basophilic stippling and the presence of clover-leaf cells, underscore the potential of Pb to cause anemia and cellular damage. Furthermore, histopathological analysis indicated that Pb exposure can damage testicular structure, reduce spermatogenic cell populations, and lead to seminiferous tubule atrophy. These findings suggest that although Pb levels remain within tolerable limits, long-term exposure can result in serious consequences for the reproductive health of stray cats, with implications for the overall ecosystem and animal health.

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Ethical Considerations

Ethical approval was obtained from the Faculty of Veterinary Medicine, Universitas Airlangga (approval number: 1.KEH.114.07.2023), ensuring that all procedures conformed to animal welfare standards.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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