

ORIGINAL ARTICLE

HISTOPATHOLOGICAL EVALUATION OF WISTAR RATS EXPOSED TO PARTICULATE MATTER POLLUTED AMBIENT AIR OF PORT HARCOURT METROPOLIS, RIVERS STATE, NIGERIA

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ABSTRACT**Background:**

Globally, air pollution constitutes a significant threat to human health affecting various organs of the body.

Objective:

This study evaluated the histopathological changes in respiratory, renal, and cardiac tissues of the Wistar rats exposed to particulate matter (PM) polluted ambient air (indoor and outdoor).

Methods:

Twenty Wistar rats were imported from the University of Nigeria, Nsukka (non-PM polluted environment). Four rats were immediately sacrificed before the commencement of the study to serve as a control group. The remaining 16 rats were grouped into two (indoor and outdoor) (n=8), then left for 90 days. At the end of the 90 days, the rats were euthanized using diethyl ether, then the lung, hearts, and kidneys were harvested for histopathological studies.

Results:

Indoor and outdoor PM exposure showed thickening of inter-alveolar septa and shrinking of alveoli sacs in the lungs. Fused peripherally placed nuclei, branched, weaved, and reunited cardiac myofibril were observed in the tissues from the indoor and outdoor exposed groups. Occlusion of bowman space was observed in the outdoor PM exposed group.

Conclusions:

PM exposure in River state was associated with marked distortion of the histo architecture of the lungs, heart, and kidney among the experimental animals.

Keywords: Particulate matter, Air pollution, Histopathological alteration, Port Harcourt metropolis

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Conflict of Interest: None is declared

INTRODUCTION

Particulate matter pollution is said to result from emissions due to incomplete combustion from industries, vehicles, heavy-duty, and power-generating machines, and other petrogenic and pyrogenic activities (1,2). These particles, especially fine particles less than 10 μ m (2.5 and 10) in aerodynamic diameter enter the human system primarily through respiration, distorting the 12 kg of clean air required for daily survival and causing diseases and death (3).

Globally, air pollution constitutes a significant threat to human health (4). About 4.2 million premature deaths occur annually due to ambient air pollution, with particulate matter contributing principally to air pollution (4). More than 90% of people live in areas with contaminated ambient air leading to the loss of about 7 million lives annually (5). Air quality is observed to be improving in developed nations, due to the zero-carbon emission policy, the use of electric cars, and awareness of tree planting (6). However, this is not the case in the cities of low-and middle-income nations, Nigeria and Port Harcourt metropolis inclusive (6). Residents in oil and gas and petrochemical-contaminated environments have been noted to be at risk of air pollution and its associated health effects (3,7). With Port Harcourt being Nigeria's petroleum and petrochemical hub, the presence of particulate matter air pollution remains a concern to residents.

The deteriorating state of air quality in Port Harcourt is largely due to continuous legal and illegal exploration and refining of crude oil and petrochemicals (8), gas flaring and open municipal waste burning from dump sites, tyre burning among others (7). These activities resulted in the sudden appearance of dark and sticky soot haze that stains cloths and surfaces, palms, feet, and the nostril and

occludes the visibility of residents (9) and health concerns of the populace, especially the vulnerable remains worrisome (10).

The health effects of airborne particulate matter have been epidemiologically and toxicologically documented with notable increase in trends of health risks. Acute and chronic morbidities such as cardiopulmonary, renal, skin diseases, obstetric abnormalities, carcinogenic observations, and mortality associated with particulate matter pollution have been implicated in several studies (3,10). Continuous 10 μ g/m³ increase in outdoor air pollution (PM₁₀) have been found to reduce life expectancy by 0.64 years (11). Approximately 15% of deaths due to air pollution could be mitigated by reducing PM₁₀ pollution from 70 to 20 μ g/m³ and annual PM_{2.5} from levels 35 to 10 μ g/m³ (12). Therefore, the present study aimed to evaluate the histopathological changes in respiratory, renal, and cardiac tissues of Wistar rats exposed to outdoor and indoor ambient air.

METHODS

Experimental Design Using Animal Model

Animal model researches are often utilized when the possibility of using human subjects is rather slim or not visible due to ethical or research factors. This way, the research on animals was used to infer the findings to raise actions and recommendations. In this experimental study, exposure (soot) is defined prior to development of outcome (effect on tissue), hence temporal sequence is established (13–15). For this study, Wistar rats (not more than one month old) from non-soot polluted environment was imported and exposed to soot polluted environment in Port Harcourt over a period (90 days) and differences in tissue architecture examined (Fig.1).

Measurement of Indoor and Outdoor PM Levels

For the entire experimental period the level of particulate matter of the environment was monitored using a Handheld Suspended

Particulate Matter optical meters (China Way CW-HAT200). This was done in the morning, afternoon, and evening; and was reported as to be within the ranges of 32 $\mu\text{g}/\text{m}^3$ to 467 $\mu\text{g}/\text{m}^3$ (PM_{2.5}: indoor = 32–291.5 $\mu\text{g}/\text{m}^3$ [median; outdoor = 79.75, indoor = 48.75] and PM₁₀: 67.5–467 $\mu\text{g}/\text{m}^3$ [median; outdoor = 150.00, indoor = 114.25]). The reported values of particulate matter levels in Port Harcourt were far above WHO acceptable limit, therefore suggest increased possibility of human exposure (16).

Animal Housing and Care

Twenty Wistar rats (4 weeks old rats, weighing between 25–30 grams) were obtained from the University of Nigeria, Nsukka (an environment free of petroleum based activities, with PM_{2.5}: 1–12 $\mu\text{g}/\text{m}^3$ and PM₁₀: 4–25 $\mu\text{g}/\text{m}^3$) (16). The obtained Wistar rats were transported to the animal house of the Department of Human Anatomy, Rivers State University, Port Harcourt for the experimentation with PM_{2.5}: 32–291.5 $\mu\text{g}/\text{m}^3$ and PM₁₀: 33–467 $\mu\text{g}/\text{m}^3$. The experimental animals were housed in a well-ventilated cage under controlled experimental setting. The experimental animals were allowed unrestricted access to food and clean water. The experimental rats were allowed to acclimatize for two weeks before the commencement of the study. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Research and Ethics Committee of Rivers State Ministry of Health (MH/PRS/391/Vo.2/632 on 13 February 2020).

Animal Exposure and Treatment

Following the guidelines of the Institutional Animal Care and Use Committee (IACUC), 4 randomly selected rats were humanely sacrificed following administration of the diethyl ether on arrival, therefore serving as the baseline. The remaining 16 rats were divided into two groups; indoor (8 rats) and outdoor (8 rats). The indoor group animals were left indoor for the entire experimental period (90 days), while the outdoor animals were brought out to the open ambient for 8 hours (8 am–4 pm) every day throughout the experimental period (90 days).

Animal Sacrifice

At the end of the 90 days, the experimental animals were humanely sacrificed using diethyl ether. A transabdominal incision was done to expose the lungs, heart and the liver for harvesting. The harvested organs were fixed in 10% neutral buffered formalin.

Tissue Processing

The fixed lungs, heart and the kidney tissues were trimmed, then processed with the aid of an automated vacuum tissue processor (TP-1020) in the Histological Unit of the Department of Human Anatomy, College of Medicine Rivers State University. The processed tissues were cut with the aid of a rotary microtome at 6 μ . The cut tissue sections were stained using hematoxylin and eosin (H & E) as described by Usman et al. (17) and Usman et al. (18). The stained tissues slides were examined with the aid of an Olympus CX43 microscope. The photomicrographs were taken using an Amscope mounted on the microscope, at 400 magnifications.

RESULTS

Histopathological assessment of the lungs

The photomicrograph from the control group (rats from non PM polluted environment) show normal lung section with intact bronchiole lined with psuedostratified columnar epithelium, thin inter-alveolar septa (IAS), alveoli sacs (AS), and congested blood vessels (BV) (Figure 2). Lung section from the group exposed in a polluted environment (indoor) showed marked histological distortion, thick inter-alveolar septa (IAS) and shrunken alveoli sacs (AS) (Figure 3). Lung section from the group exposed in a polluted environment (outdoor) showed marked histological distortion, thick inter-alveolar septa (IAS) and shrunken alveoli sacs (AS) (Figure 4).

Histopathological evaluation of the heart

The photomicrograph of heart section from the control group showed intact cardiac myofibril (Mf) and centrally placed nuclues (Nu) (Figure 5). The photomicrograph of heart section from the group exposed in a polluted environment (indoor) showed marked histological distortion peripherally placed nuclei (Nu) and branched, weaved and reunited cardiac myofibril (Mf) (Figure 6). The photomicrograph of heart section from the group exposed in a polluted environment (indoor) showed marked histological distortion; fused peripherally placed nuclei (Nu) and branched, weaved and reunited cardiac myofibril (Mf) (Figure 7).

Histopathological evaluation of the kidney

Kidney section from the control and group exposed in a polluted environment (indoor) showed intact histo-architecture renal tubule (T), patent bowman's capsule (C), and tuft glomerulus (G) (Figure 8 and 9 respectively). On the other hand rats from the group exposed in a polluted environment (outdoor) showed region with occlusion of bowman space (Figure 10)

DISCUSSION

Exposure of the experimental rat to PM polluted environment (both out and indoor) were marked with

distorted histo-architecture of the lungs presenting as thickening of inter-alveolar septa and shrunken alveoli sacs. Sections from the heart of exposed rats (both out and indoor) revealed the heart was not left out of the harmful effect of PM exposure during the period of the experiment when compared with sections from the control group. On the other, kidney sections from the exposed group (outdoor) revealed region with occlusion of bowman space. Our finding correlated with the reports that exposure to PM in air polluted environment was associated with an upsuges in cardiovascular, respiratory and renal dysfunction, with consequent increase in mortality (19,20).

There are various suggestion on possible mechanism via which inhaled PM exerts their damaging effects on the various tissues of the body, including the lung, heart, and kidney (21). The direct and indirect mechanism were recognized (21). For the direct pathway, PM translocate into the blood stream, then finally the target organ where the impacts of PM is felt (22). The indirect pathway is mediated by inflammatory response and pulmonary oxidative stress (22).

PM at the level of the lung epithelial are able to elicit oxidative and inflammatory responses (22). Oxidative imbalance and inflammatory cascades in the lung has the potential to interfere with the lung respiratory function (23). In some instance could cause respiratory distress in man and experimental animal models (24). The size and chemical composition of PM make it much easier for this substances to interact with the epithelial layer of the alveoli, consequently crossing the lung blood barrier (25). The movement of PM across this barrier make it easy for PM to reach the different organs of the body (21). PM are able to reach the different organs of the body even at a low concentration (26–28). Once PM gets into the blood stream, they could be deposited in the cardiovascular epithelial of the heart and the blood vessels consequently aggravating local oxidative stress and inflammation, with resultant atherosclerotic plaque instability forming thrombus (29). Studies using experimental animal revealed premature ventricular beat and increased ejection fraction following intravenous injection of PM isolated from ambient air (22).

For the indirect pathway, the oxidative stress and activated

inflammatory pathway in lungs due to exposure to PM plays very important role (21). PM exposure can trigger inflammatory response when deposited in the lung (30–32), therefore increasing the level of circulating pro-inflammatory cytokines such as C-reactive protein (CRP), Interleukin 1 β (IL-1 β), Interleukin 6 (IL-6), and Interleukin 8 (IL-8) (33–36). Systemic inflammatory response is a major risk factor for atherosclerosis progression. CRP, IL-1 β , IL-6, and IL-8 have been linked with increased blood coagulation incidence and endothelial dysfunction, with resultant exacerbation of myocardial ischemia. Reactive oxygen species dependent mechanism have been shown to be involved in the PM particulates triggered pro-inflammatory pathway (34). Study by Gurgueira et al., (34) revealed increased amounts of ROS in the experimental animals lung and heart PM exposure. ROS has also been shown to be linked to vascular dysfunction, myocardial injury, atherosclerosis, and cardiac arrhythmias (19,37).

The presence of region with marked occlusion of the bowman space suggests possible effects of PM exposure (16) in the animal models used in the present studies. Our observation is in line with the finding of Huang et al., (30) who reported that PM exposure in experimental animals was associated with renal injury, marked with edema of renal tubule epithelia, shrinkage of glomerulus, and capillary congestion. The effect of PM on the kidney histo-architecture may not be unconnected to the cardinal role the kidney play in detoxification and excretion of toxic waste (39).

CONCLUSION

The finding from the present study implicates possible histo-pathological changes in the lungs, heart, and kidney associated with PM exposure in Port Harcourt. This therefore makes this study timely, as this could enable extrapolation of the possible impact of PM exposure in human especially among those living within this region. However, the authors recommend the conduct of the same study in a more controlled environment, which the authors could not achieve due to resource limitation. Other study should consider possible deployment of intra-tracheal

instillation procedure.

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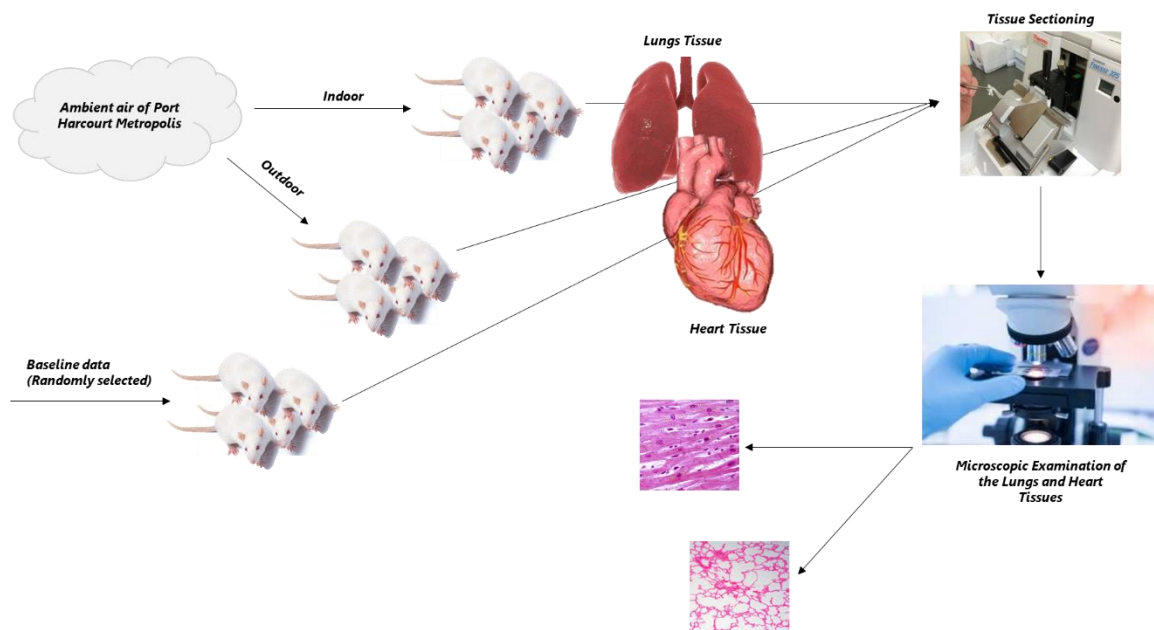


Figure 1: Animal experimental model

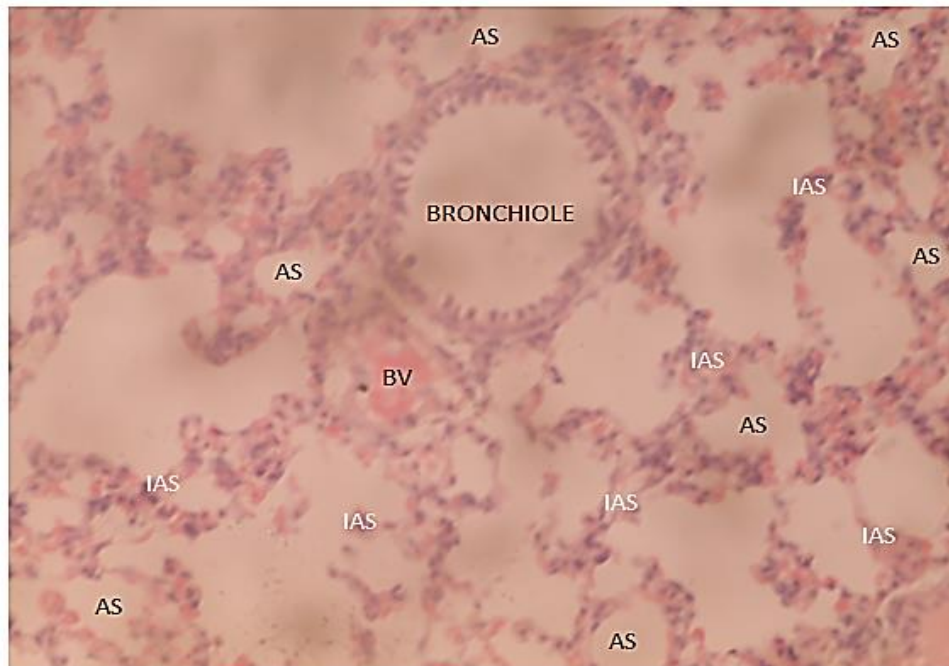


Figure 2: Normal lung section from the control group, showing the bronchiole lined with psuedostratified columnar epithelium, thin inter-alveolar septa (IAS), alveoli sacs (AS), and congested blood vessels (BV) (Mag X400 H&E).

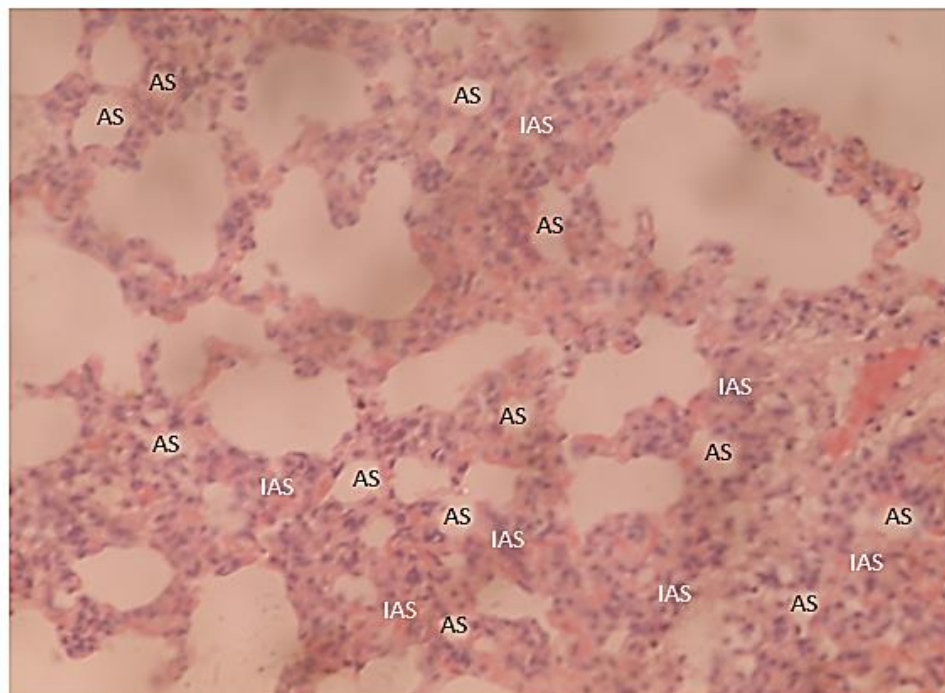


Figure 3: Lung section from the group exposed in a polluted environment (indoor). Showing marked histological distortion, thick inter-alveolar septa (IAS) and shrunkened alveoli sacs (AS) (Mag X400 H&E).

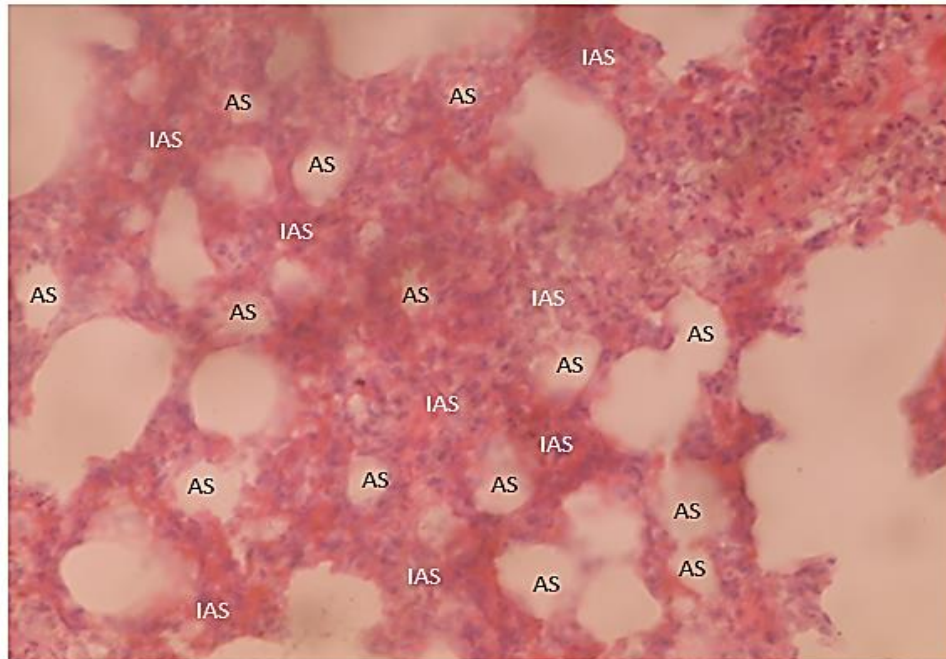


Figure 4: Lung section from the group exposed in a polluted environment (outdoor). Showing marked histological distortion, thick inter-alveolar septa (IAS) and shrunken alveoli sacs (AS) (Mag X400 H&E).

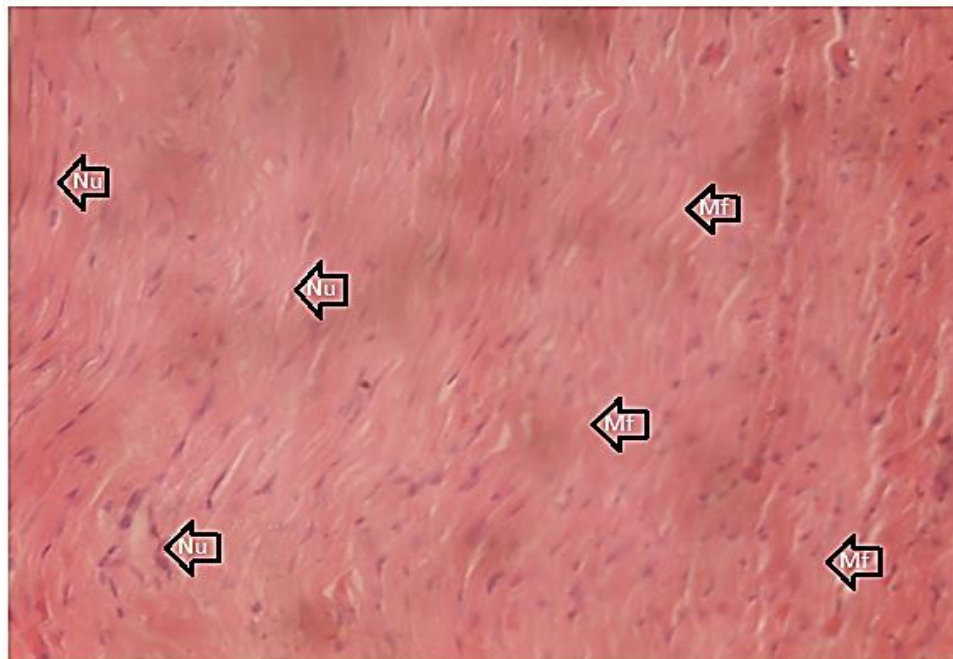


Figure 5: Normal heart section from the control group, showing intact cardiac myofibril (Mf) and centrally placed nuclues (Nu) (Mag X400 H&E).

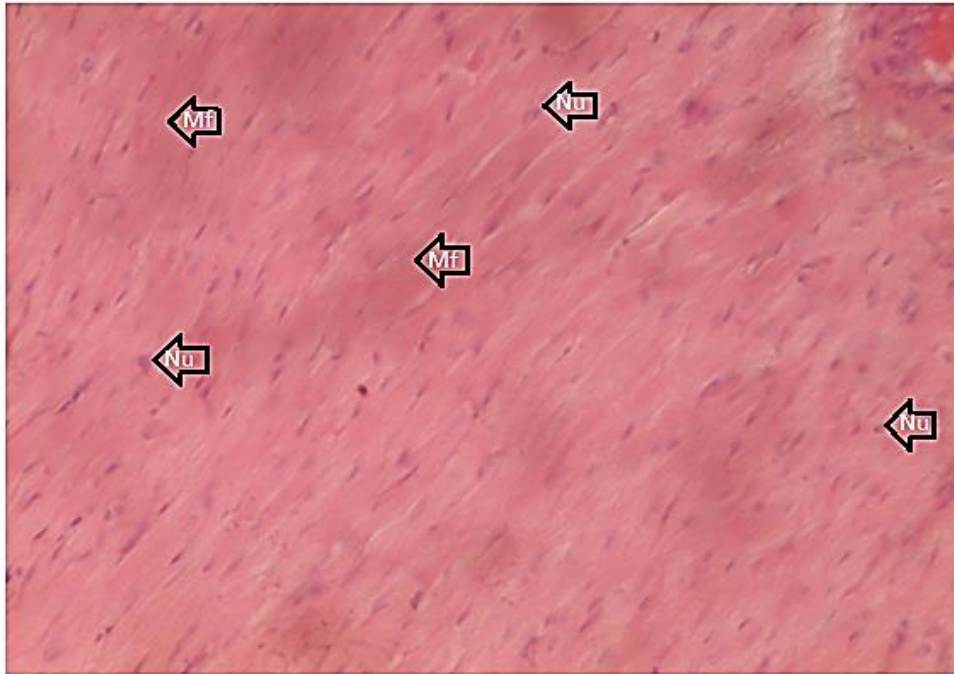


Figure 6: Cardiac muscle section from the group exposed in a polluted environment (indoor). Showing marked histological distortion peripherally placed nuclei (Nu) and branched, weaved and reunited cardiac myofibril (Mf) (Mag X400 H&E).

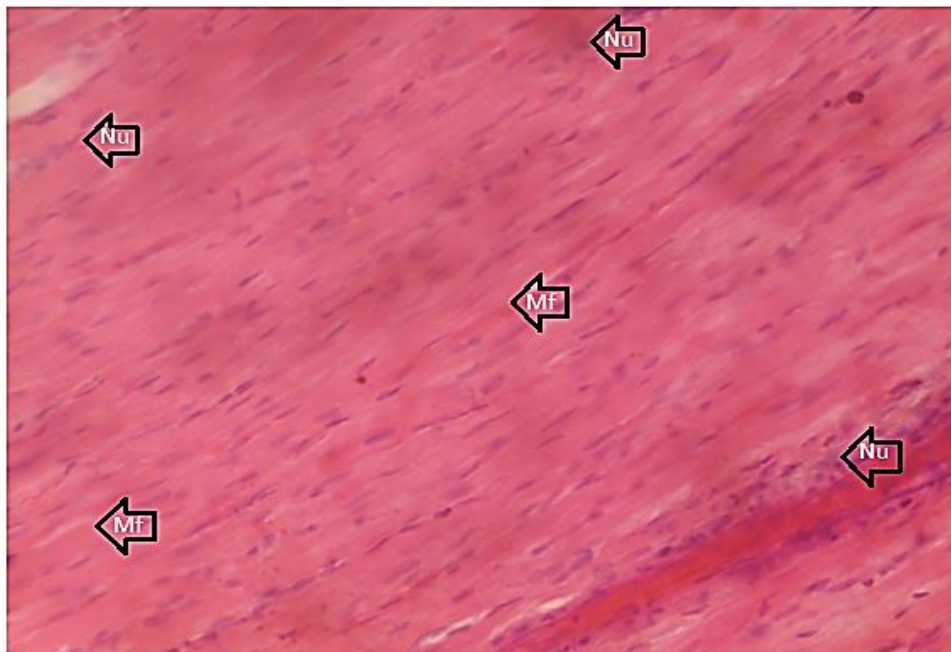


Figure 7: Cardiac muscle section from the group exposed in a polluted environment (outdoor). Showing marked histological distortion; fused peripherally placed nuclei (Nu) and branched, weaved and reunited cardiac myofibril (Mf) (Mag X400 H&E).

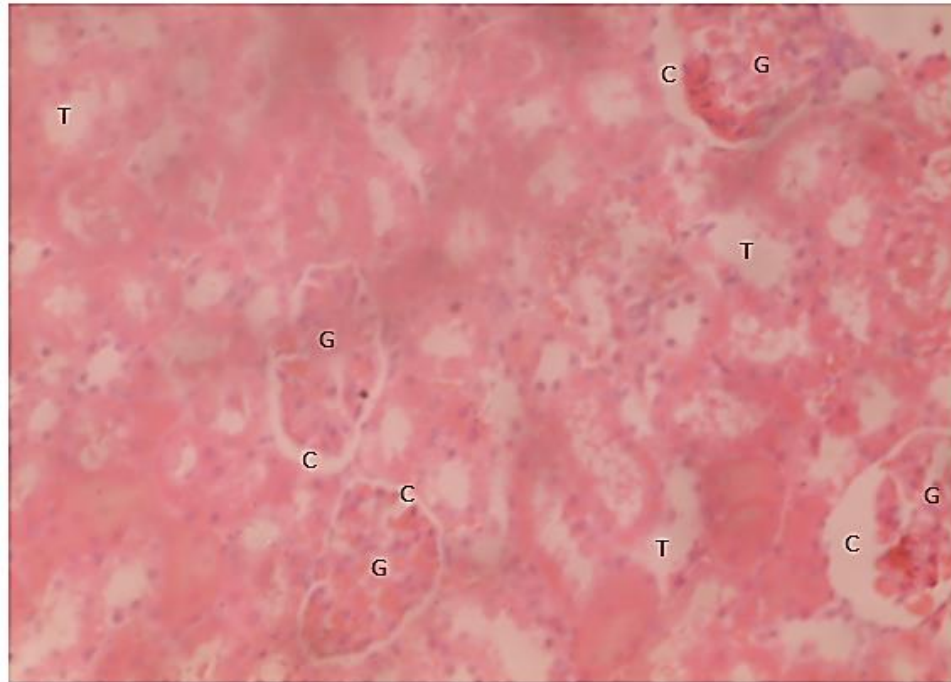


Figure 8: Kidney section from the control group. Showing intact histoachetechture renal tuble (T), patent bowman's capsule (C), and tuft glomerulus (G) (Mag X400 H&E).

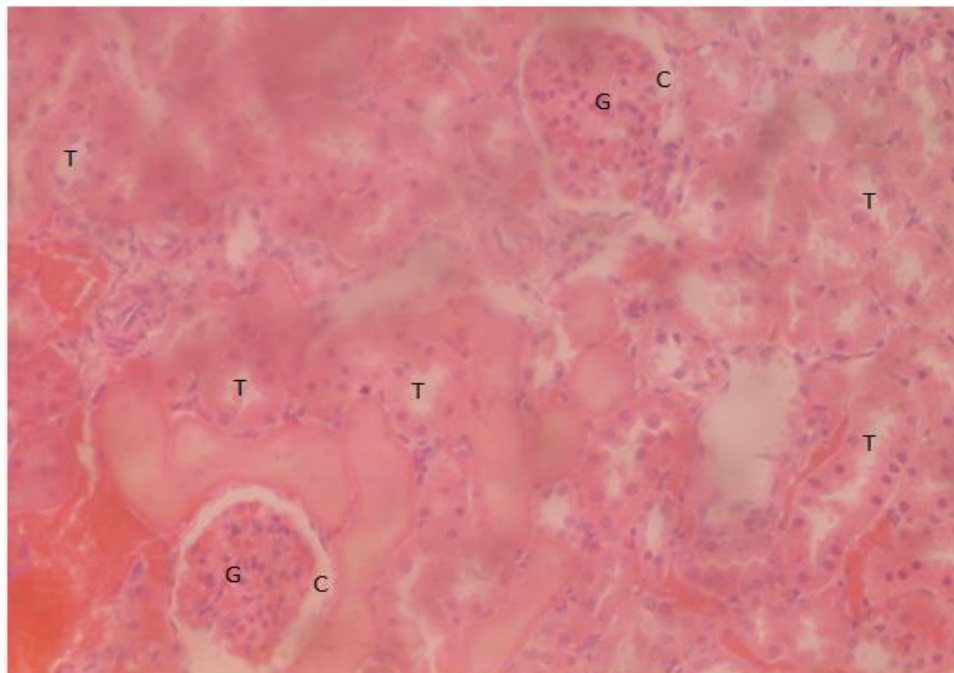


Figure 9: Kidney section from the group exposed in a polluted environment (indoor). Showing intact histoachetechture renal tuble (T), patent bowman's capsule (C), and tuft glomerulus (G) (Mag X400 H&E).

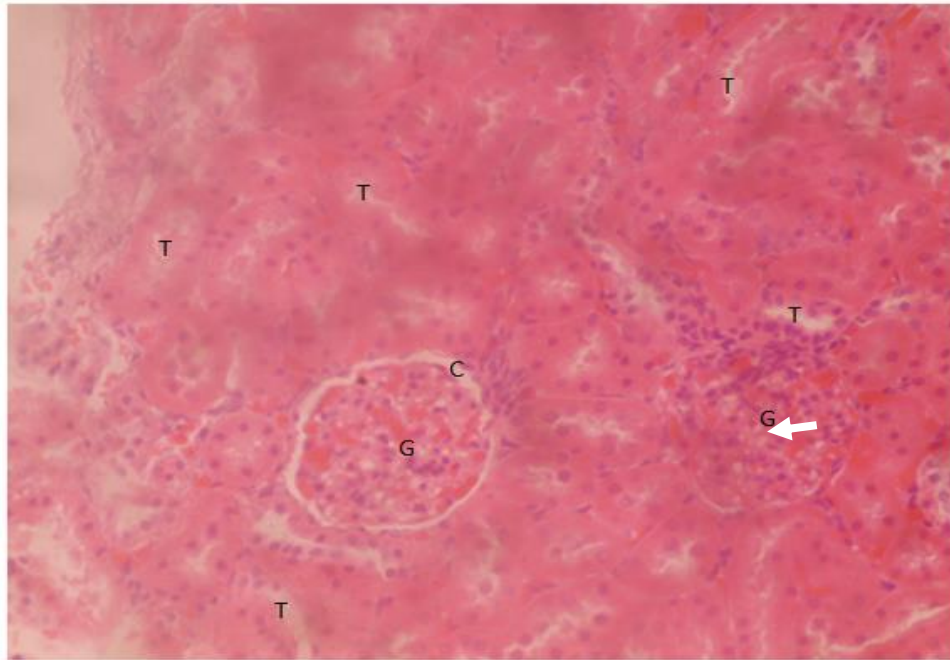


Figure 10: Kidney section from the group exposed in a polluted environment (outdoor). Showing distorted histoachetechture renal tube (T), patent bowman's capsule (C), occuluded bowman space (white arrow) and tuft glomerulus (G) (Mag X400 H&E).