Journal of Himalayan Earth Sciences Volume, No., 2026 pp. 1-25

Comparative Assessment of Airborne Pollutants and Occupational Exposure in Poultry Houses across Multiple Cities in Pakistan

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Submitted Date:11/10/2026 Acceptance Date: 04/11/2026 Publication Date:---------

Abstract

Poultry farming is a significant industry in Pakistan. However, evidence regarding air quality within poultry houses and associated occupational risks remains limited. This study evaluated air pollutants and heavy metals for 5 farms located in Lahore, Layyah, Multan, Faisalabad, and Islamabad during summer and winter months. Concentrations of CO_2, CO, NO_2, NH_3, $PM_{2.5}$, and PM_{10} were measured using a HAZ-SCANNER HIM 6000, and temperature, humidity, and wind speed were measured by a digital hygrometer and anemometer. To determine levels of lead and cadmium, dust was analysed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) and workers' nails were analysed by Flame Atomic Absorption Spectrophotometry (FAAS). The concentrations of air pollutants varied significantly across sampling sites and seasons, with higher concentrations in shed centres and litter/manure areas during winter ($p < 0.001$). Seven locations per farm were monitored on three occasions in each of June, July, November and December, yielding 420 readings per air-quality variable. The study included 60 dust samples from one location per farm and nail samples from ten workers, including five smokers and five non-smokers. An exploratory repeated-measures analysis evaluated sampling location and month using five farms as subjects and the mean of three readings per farm-location-month. Winter mean concentrations exceeded summer means for NO_2 (0.0482 ± 0.0315 versus 0.0271 ± 0.0118 ppm), CO_2 (2369.91 ± 828.13 versus 2149.46 ± 763.05 ppm), CO (15.63 ± 4.45 versus 8.69 ± 1.73 ppm), $PM_{2.5}$ (30.76 ± 18.49 versus 24.66 ± 11.24 µg/m³) and PM_{10} (131.29 ± 81.43 versus 96.94 ± 52.20 µg/m³). NH_3 averaged 25.95 ± 14.30 ppm in winter and 24.22 ± 12.17 ppm in summer, although the monthly effect was not statistically significant. NO_2, CO, NH_3, $PM_{2.5}$, and PM_{10} exceeded WHO reference limits at several locations. NH_3 exceeded the OSHA limit, and other gaseous pollutants did not exceed the OSHA limit. The concentrations of Pb had significant seasonal differences ($p < 0.001$), while Cd differed significantly with time ($p = 0.001$). Mean nail Pb and Cd concentrations were descriptively higher among smokers; these preliminary differences do not establish a correlation between smoking-related and occupational effects. The findings characterise the selected farms and support further investigation using replicated farm sampling and personal exposure monitoring. The study fills an important research gap by offering a multi-city and seasonal evaluation of air pollution and possible exposure of individuals working in the poultry houses in Pakistan. The results are consistent with the need to advocate for better ventilation, regular air quality monitoring, on-site health surveillance and the use of personal protective equipment to reduce exposure and potential risk to workers' health.

Keywords: Poultry farms; Air quality; Seasonal variation; Dust metals; Exploratory bio-monitoring.

1. Introduction

Demand for food has increased markedly with global population growth in recent decades. Consequently, between 1980 and 2020, global egg production tripled, from 26.2 million to 86.7 million metric tonnes (Guyonnet, 2023). Globally, animal

production has changed over the last 50 years from extensive and semi-intensive systems to fully intensive systems; Pakistan's poultry industry is also following this trend. This change has allowed greater productivity and efficiency but also elevated the vulnerability of farm animals to disease outbreaks, particularly in restricted production environments (Gržinić et al., 2023).

Population growth has increased demand for food, encouraging intensive egg and meat production. Intensive poultry production may be a significant source of air pollution because such systems contain several thousand to several hundred thousand birds in one or more buildings. This is leading to growing concerns about the emission of pollutants, including toxic gases and airborne particles or dust, from concentrated poultry operations, especially concerning environmental and human health impacts. Airborne particulate pollutants are present in poultry houses for much of the work shift (Górny et al., 2023).

Recent studies have associated poor air quality with respiratory diseases and increased mortality among the population. In 2019, 99% of the global population breathed air that did not meet the World Health Organization's standards. In addition, 4.2 million people died in 2016 from poor air quality, of which 91% occurred in low- and middle-income countries, including Southeast Asian countries and the West-Pacific region (Baccarelli et al., 2011; World Health Organization, 2018, 2024). Extensive amounts of ammonia, particulate matter, and bacterial aerosols will be emitted during poultry production processes. Particles are a major source from dried manure in animal housing facilities. Particle emission factors vary according to the type of waste, particularly in poultry facilities for PM_{10}. Typically, higher for compost than for droppings. In poultry environments, dust levels can be as high as 8% of dried faeces. Particles can be classified as total suspended particulate (TSP), PM_{10} (which are < 10 μm in aerodynamic diameter), or $PM_{2.5}$ (which are < 2.5 μm in aerodynamic diameter); when inhaled, they may cause tracheitis, Bronchial inflammation, Pulmonary emphysema, and other diseases (Post et al., 2021).

Particulate matter (PM) is known to have harmful effects on human health because of its association with respiratory and cardiovascular diseases (Macchi et al., 2023). Further, it has negative impacts on animal health and productivity (Emert et al., 2023; Wang et al., 2023). PM from animals typically contains a variety of harmful compounds, such as various components of smell, heavy-metal elements, germs, viruses, and endotoxins (Li et al., 2021). These substances can be widely dispersed and may lead to pathogenic and allergic reactions at the local community level (Dai et al., 2020). The livestock sector has developed fast and is now a significant source of PM emissions, a threat to human health and air quality.

Particularly in countries where the air quality is already low, the potential health impacts are significant. According to the IQAir World Air Quality Report 2024, Pakistan was ranked the third most polluted country in the world, with an annual mean $PM_{2.5}$ level of 73.7 μg/m³, which stands at 14.7 times the annual guideline by WHO (5 μg/m³). Lahore had an annual $PM_{2.5}$ concentration of approximately 102.1 μg/m³, which highlights the high level of background air pollution in major cities of Pakistan (IQAir, 2025). Lahore, Peshawar, Faisalabad, Islamabad, and Rawalpindi were among the major Pakistani cities with significant $PM_{2.5}$ concentrations each year.

In winter, poor ventilation is often associated with accumulation of indoor pollutants such as carbon dioxide (CO_2) and ammonia (NH_3) that can reach harmful concentrations and cause respiratory diseases, adversely affecting the health and productivity of chickens (Zheng et al., 2020; Tong et al., 2020). Furthermore, the current suboptimal ventilation structure pattern still makes the distribution of indoor air parameters in bird structures very Irregular and uneven from the longitudinal centre. This non-uniformity may result in areas of poor air exchange, and these are often called “ventilation dead zones”. These areas are at higher risk of cold stress and are areas of localised high pollutant levels (Tong et al., 2019; Wang et al., 2019; Al-Assaad et al., 2021).

The main indoor and outdoor pollutants from abattoirs and livestock farms are identified as fly ash, particulate matter, nitrogen dioxide, sulphur dioxide, carbon monoxide, hydrogen sulphide, ammonia and methane (Magaji and Hassan, 2015). The pollutants are responsible for an estimated 3.3 million deaths worldwide. Children under 5 years of age and adults older than 80 years are especially vulnerable to deaths caused by air pollution in developing countries. The pollution of heavy metals is common in all parts of the world, especially in the vicinity of urban regions and industrial facilities (Zhang, 2023).

On the other hand, toxic components like lead (Pb), chromium (Cr), mercury (Hg), nickel (Ni), and cadmium (Cd) are frequently associated with pollution and can be harmful to living organisms when their levels exceed a certain threshold (Mohajane, 2022). The measurement of heavy metals in dust from poultry houses provides information on the contamination status of the entire poultry farm but does not necessarily provide information about the internal exposure of individual workers. Bio-monitoring is therefore used as an adjunct to assess occupational exposure to potentially harmful metals to fill this need. Human nails have been used as a non-intrusive biological matrix for the identification of exposure to heavy metals. The presence of Pb and Cd in human biological samples, including nails, has been confirmed by recent studies in Pakistan.

Pakistan is the 11th largest poultry-producing country, and the poultry industry contributes a major chunk in the meat industry in the country (Government of Pakistan, 2025). The poultry sector is of significant importance and is still expanding in Pakistan. According to the Pakistan Economic Survey 2024-25, there were around 2.58 million tonnes of poultry meat, 26.70 billion eggs produced, and 2.26 billion poultry birds in 2024-25, assuming the commercial broiler production was around 2.06 billion birds. Currently, the country produces approximately 1.55 billion broiler chickens each year.

The adoption of the environmentally controlled house (ECH) has now become common in the poultry industry in Pakistan. These ECHs ensure improved feed efficiency, enhanced growth performance, superior carcass characteristics and effective disease management in poultry birds (Rahman et al., 2020). The importance of understanding air quality conditions in poultry production systems is further emphasised by the continued expansion of this sector, as well as the increasing adoption of controlled housing of poultry.

Environmental assessment in Pakistan may refer to the National Environmental Quality Standards (NEQS) for ambient air established under the Pakistan Environmental Protection Act. The applicable NEQSs contain the limits for the pollutants PM_{10}, $PM_{2.5}$, NO_2/NO_x, SO_2, CO, and lead. 24-hour limits of 150 µg/m³ for PM_{10}, 35 µg/m³ for $PM_{2.5}$, 80 µg/m³ for NO_2, and 5 mg/m³ for CO are set by the NEQS; these are ambient air limits.

In Pakistan, the poultry industry is expanding, ambient air pollution levels are high, and there are significant knowledge gaps. There is very few data exist to determine the levels of CO_2, CO, NO_2, NH_3, $PM_{2.5}$, and PM_{10} in the poultry houses of Pakistan and it is not available in all space and time. Very few studies have quantified several gaseous pollutants, particulates, and heavy metals in the same subjects, and the nail bio-monitoring data are available for Cd and Pb, and the health symptoms comparison data is limited between smoking and non-smoking workers. These gaps hinder efforts in identifying high-risk areas and worker groups. The present study aimed to assess the quality of the work environment of chicken farms in five major cities of Pakistan. The aims were to: (i) quantify CO_2, CO, NO_2, NH_3, $PM_{2.5}$ and PM_{10}; (ii) assess the spatial and seasonal variation; (iii) determine the concentration of metals in dust collected from the poultry house and in fingernails of workers; (iv) compare the levels of metal exposure between smoking and non-smoking workers; and (v) compare selected health symptoms between the smoking and non-smoking workers. This study has included environmental monitoring, heavy metal assessment, bio-monitoring, and health information, which gives a complete picture

of occupational exposure in the poultry industry in Pakistan.

2. Materials and Methods

2.1. Study Location

The study was conducted at selected poultry farms in the agricultural areas of Lahore, Faisalabad, Multan, Layyah and Islamabad, Pakistan. These cities have different geographical and climatic conditions. Lahore is present at approximately 31.52°N, 74.36°E Faisalabad at 31.45°N, 73.14°E, Multan at 30.16°N, 71.52°E, Layyah at 30.96°N, 70.94°E and Islamabad at 33.68°N, 73.05°E. The present study cities generally have hot summers and comparatively cooler winters, and Islamabad has a relatively cooler and humid climate. The Lahore, Layyah and Multan average annual temperatures are approximately 24.0°C, 25.2°C, and 25.6°C, respectively, whereas the average annual temperatures of Faisalabad are 26.2°C, and Islamabad is 21-22°C. Figure 1 shows the geographical locations of farm positions. Table 1 summarises the characteristics of the farms.

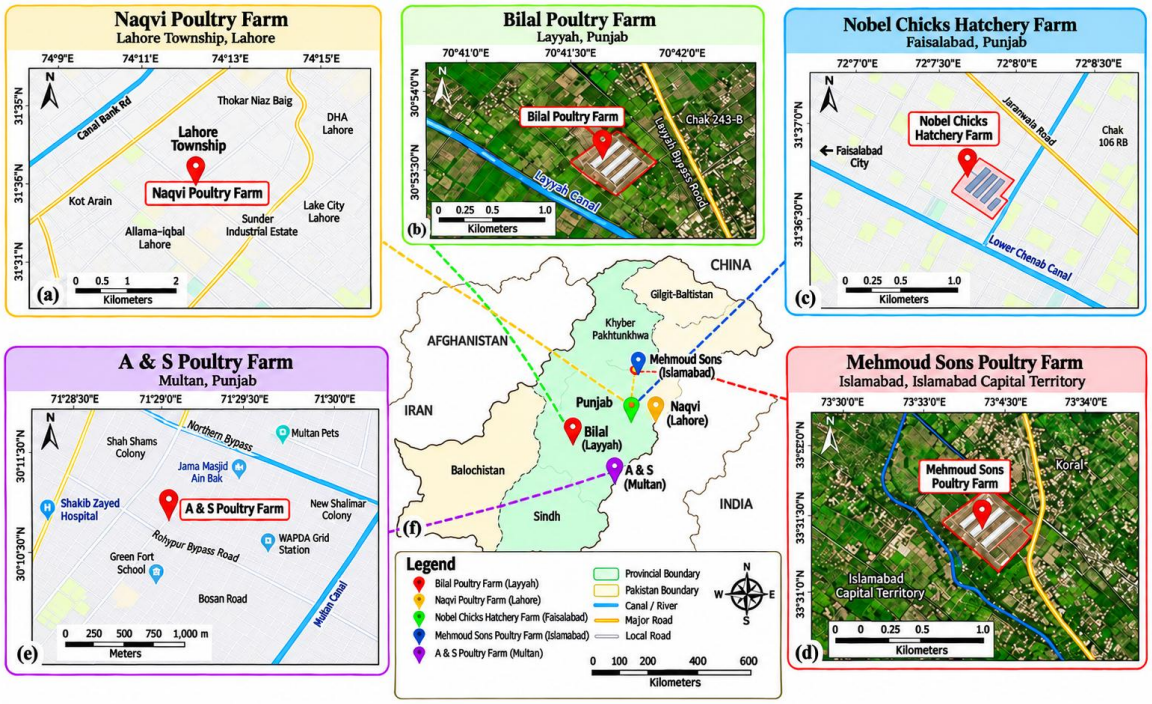

Fig. 1. Approximate geographical locations of the five study cities

Table 1: Characteristics of the five selected farms

Parameter	Lahore	Layyah	Multan	Faisalabad	Islamabad
Coordinates	31.52°N, 74.36°E	30.96°N, 70.94°E	30.16°N, 71.52°E	31.45°N, 73.14°E	33.68°N, 73.05°E
Poultry farm	Naqvi Poultry Farm	Bilal Poultry Farm	A & S Poultry Farm	Noble Chicks Poultry Farm	Mehmood Sons Poultry Farm
Bird capacity	15,000	10,000	16,000	18,000	12,000
Ventilation type	Natural + fans	Tunnel	Natural + fans	Tunnel	Tunnel
Litter type	Wheat straw	Rice hulls	Sawdust	Rice hulls	Wheat straw

2.2. Sampling Design

One farm was purposively selected in each city, giving five farm units. The seven monitoring points recorded were: feeding operation, birds 1-25 days, birds 26-50 days, shed centre, litter/manure area, exhaust fan, and outdoors. Three readings were taken every other day at each site in June, July, November, and December. Thus, the number of records in the air dataset is 5 farms × 7 locations × 4 months × 3 readings = 420 records for each variable. Because only one farm was sampled in each city,

differences among "cities" in this study were the differences among the five studied farms and cannot be generalised to the poultry industry of the whole city. Likewise, the three readings taken at a site within a month are repeated measurements of the same location, not independent replicates; this was accounted for in the statistical model. June and July were classified as summer and November and December as winter. The characteristics of the selected poultry farms are summarised in Table 1.

2.3. Sampling Positions, Timing and Air-Quality Measurements

The measurements were taken during the peak activity time of the poultry houses, at an altitude of about 1.5 m above the ground, between 10:00 and 18:00 pm. The instrument was placed clear of walls and the direct airstream of the fans. Three readings were obtained at each location each month on alternate days.

The instruments used were a HAZ-SCANNER HIM-6000 (Environmental Devices Corporation, USA) for CO_2, CO, NO_2, NH_3, $PM_{2.5}$, and PM_{10} measurements. A digital hygrometer and anemometer were used for measuring temperature, relative humidity, and air speed. The instrument was calibrated and verified by the institutional laboratory as suggested by the manufacturer. Zero and span calibration was performed and about 15 minutes of warm up prior to measurement. The gas concentration is expressed in ppm, particulate concentration in µg/m³, temperature in °C, relative humidity in %, and air speed in km/h. Exhaust measurements were taken about 1 m from the fan outlet and outdoor measurements were taken about 20 m upwind of the poultry shed. Wind speed was measured in km/h.

2.4. Analysis of Potentially Toxic Metals in Poultry Dust

A total of 60 settled-dust samples were analysed (five farms × four months × three samples). Samples were collected from the shed centre in Faisalabad and Islamabad and from litter/manure areas in Lahore, Layyah and Multan. Approximately 8 g of dust was collected per sample using a clean sampling tool. Samples were placed in sealed bags and transported to the Environmental Science Research Laboratory (ESRL) at Lahore College for Women University (LCWU). Samples were dried, homogenised, and stored in airtight containers for analysis.

2.4.1. Acid Digestion

The dust samples were digested using a method adapted from Alghamdi et al. (2022). Briefly, 0.5 g of homogenised dust was transferred to a digestion vessel. Next, 10 mL of HCl was added, and the mixture was heated on a hot plate. After that, 10 mL of concentrated HNO_3 was added and heating was ongoing until complete digestion. Then, the digest was chilled, purified, and brought to a volume of 50 mL with deionised water in a volumetric flask before instrumental analysis. The calibration standards were prepared from certified stock solutions of lead (Pb) and cadmium (Cd), and reagent blanks underwent the similar digestion procedure without the sample.

Lead and cadmium concentrations in poultry dust samples were analysed using an inductively coupled plasma–optical emission spectrometry (ICP-OES) (Agilent 5110-vdc). Standard solutions of known concentrations were prepared from certified stock solutions for calibration. Each analytical batch included reagent blanks, calibration standards, and a continuing calibration check standard after every ten samples. Calibration curves were considered acceptable with a coefficient of determination (R^2) of 0.999 for both lead and cadmium. Calibration curves were prepared independently for each element (lead (Pb) and cadmium (Cd)) at their respective analytical emission wavelengths (221.35 nm and 229.42 nm, respectively). These sample solutions were aerosolised and introduced into the plasma, while the levels of Pb and Cd were determined using their calibration curves. The limits of detection (LOD) and limit of quantification (LOQ) were 0.051, 0.128 mg/kg for Pb and 0.045, 0.147 mg/kg for Cd, respectively. Analytical recoveries for both Pb and Cd were 90% and 92%, respectively. Analytical precision, expressed as the relative standard deviation (RSD), was 3.8% for Pb and 4.6% for Cd, determined by taking five replicates per sample.

2.4.2. Quality Assurance and Quality Control for Dust Analysis

Quality assurance/quality control procedures were implemented to ensure the reliability of analytical information. All laboratory apparatuses were cleaned, rinsed with distilled or deionised water, and treated with 1% HNO_3 to reduce contamination. Samples were also run with digestion blanks (no dust sample) to aid in determining possible contamination. Instrument performance was checked using calibration and quality-control standards to monitor the performance of the instruments. The accuracy of the analytical method was assessed using NIST SRM 2583 as a certified reference material. Blank results, replicate measurements, calibration performance, and reference material results were used to carry out quality assessment.

2.5. Collection and Preparation of Biological Samples

Nail samples were collected from ten poultry workers, including one smoker and one non-smoker from each farm. Participants were aged 22-45 years, had worked at their poultry farms for at least one year, and were on duty for about eight hours per day. Nail samples from approximately 100 workers were collected from each worker using cleaned nail clippers after washing hands with metal-free soap. The nail samples were cleaned by washing the nails with Triton X-100, treated with acetone, and washed several times with deionised water. Samples were dried to constant mass and placed in clean containers to be used for digestion. Although these steps reduce surface contamination, complete removal cannot be assumed.

2.5.1. Digestion

The dried nail samples were wet digested with 10 mL of a 6:1 mixture of concentrated nitric acid and concentrated perchloric acid in a beaker and kept overnight at room temperature. The mixtures were then heated at 160–180 °C until a clear solution or white crystalline residue formed. The solution was then diluted to 50 mL using 0.1 N nitric acid.

Lead (Pb) and cadmium (Cd) in human nail samples were determined using an Agilent 280FS AA flame atomic absorption spectrophotometer (FAAS).

2.5.2. Quality Assurance and Quality Control for Nail Analysis

Reliable analytical results were obtained using quality assurance and quality control procedures. Reagent blanks were also run with samples to detect contamination during digestion and analysis. Certified stock standard solutions were serially diluted to prepare calibration curves for both Pb and Cd. The working standards were prepared using the single-element stock solutions certified by Merck (Darmstadt, Germany) at a concentration of 1,000 ug /L, but only when appropriate for the instrument and validated method. The wavelength of analysis for lead and cadmium was 273.6 nm and 238.2 nm. For Pb and Cd, the calibration range was 0.002-0.020 and 0.004-0.031. The R^2 value of the coefficient of determination for pooled nail samples was 0.998 and 0.999 for Pb and Cd, respectively. Eight blank samples were analysed to determine the limits of detection (LOD) and quantification (LOQ). For Pb and Cd, the LOD and LOQ were 0.052, 0.064 and 0.034, 0.047 ug/g^{-1}. The Pb and Cd recovery in fortified nail samples was from 88.5% to 96.1% and 89.3 to 96.4% at spiked concentrations of 0.10, 0.28, and 0.30 and 0.09, 0.16, and 0.21$\mu g/g^{-1}$ while repeatability was reported using relative standard deviation (RSD) for Pb and Cd. Calibration curves were drawn for both elements (Pb and Cd), which were measured separately at their respective analytical wavelength depending on the concentration levels of interest. The instrument was optimised according to the needs of each element, and sample concentrations were calculated from the calibration curve. Five replicate measurements were taken on each sample.

2.6. Exploratory Questionnaire Survey

Ten workers were selected, and smoking histories, work information, and self-reported symptoms were collected using a questionnaire. The following outcomes were listed: shortness of breath, chest tightness, wheezing, cough, headache, eye irritation, nasal irritation, reported lung infection, skin irritation, fatigue, reported

renal dysfunction, and cardiovascular problems. These responses do not represent clinical diagnoses, and no conclusions about occupational causation were drawn.

2.6.1. Inclusion and Exclusion Criteria

Workers aged 22-45 years with at least one year of poultry-farm employment and approximately eight working hours per day were eligible. Both smokers and non-smokers were included. Non-regular poultry workers and workers not in these categories were not included. The small purposive sample was designed to yield preliminary observations.

2.6.2. Questionnaire Scoring

A binary scoring system (Yes = 1, No = 0) was used to code each of the 12 health symptoms. The scores were combined for each participant to provide an overall health condition for each person (0 to 12), with higher scores indicating more health issues reported. Symptoms of workers were also evaluated independently and reported as percentage (%) and frequency (n) in each smoker and non-smoker group.

2.7. Statistical Data Analysis

Descriptive statistics (mean, SD, minimum and maximum) were performed for each air pollutant based on 420 measurements. IBM SPSS Statistics version 25 was used for all analyses. Seasons were defined according to the sampling months, with June-July considered as summer and November-December as winter. Three readings were collected for each farm-location-month combination and averaged, resulting in 28 repeated means per farm (7 locations × 4 months). A three-factor repeated measures ANOVA was conducted for each pollutant separately. The concentrations of pollutants were displayed in tables and comparison graphs to illustrate variation in pollutant concentrations across farms, sampling sites, and seasons. A p-value below 0.05 was considered statistically significant.

2.8. Ethics Approval and Informed Consent

The Advanced Studies and Research Board (ASRB) and the Institutional Review Board (IRB) of Lahore College for Women University (Ref. No. Reg/LCWU/263) approved the study. Informed consent was obtained from all participants before they were included in the study. Participation was voluntary, and participants were free to withdraw at any time. The study was conducted with confidentiality. Each participant provided written informed consent (for workers unable to read, the information was read aloud in Urdu and consent was recorded by thumb impression, in the presence of a witness). Prior to analysis, personal identifiers were removed. Owners or managers of each farm allowed sampling.

3. Results and Discussion

The analysis included 420 pollutant readings, with 210 observations in each season. Seasonal summaries are presented in Table 2, and exploration repeated measures ANOVA is reported in Table 3. The results are not intended to estimate pollution levels on the city-wide level, but rather to indicate the selected farm in each location.

3.1. NO_2 Concentrations at the Selected Poultry Farms

The results of NO_2 concentration showed a significant increase across the three days of measurement in all three interactions (Day 1: 0.0303 ± 0.0207; Day 3: 0.0448 ± 0.0294; $p < 0.001$). There was no significant difference between the different cities ($p > 0.05$), with Lahore having the highest descriptive mean concentration (0.0422). The sampling site had a significant effect on NO_2 concentration, with the highest mean being at the centre of the shed (0.0519) and the lowest at the outdoor site (0.0077), followed by the litter/manure area. There were also significant differences between months, with December (0.0485) and November (0.0479) having the highest concentrations, followed by July (0.0283) and June (0.0259). Significant Month × Site ($F(18, 112) = 8.49$, $p < 0.001$) and Day × Month × Site interactions ($F(26.292, 163.593) = 1.88$, $p = 0.009$) further indicated variations across sampling locations and measurement periods.

The concentration of NO_2 showed a positive correlation with relative humidity ($r = 0.367$, $p < 0.01$) and a negative correlation with temperature ($r = -0.240$, $p < 0.01$) and wind speed ($r = -0.252$, $p < 0.01$).

Table 2: Seasonal pollutant concentrations

Parameter	Season	N	Minimum	Maximum	Mean ± SD	Standard / guideline	Value
NO_2 (ppm)	Summer	210	0.0020	0.0570	0.0271 ± 0.0118	WHO air quality guideline (health-based) OSHA PEL (Workplace)	25 µg/m³ (≈0.013 ppm) (24 h) 9 mg/m³ / 5 ppm (Ceiling)
	Winter	210	0.0040	0.2150	0.0482 ± 0.0315		
CO_2 (ppm)	Summer	210	463.00	3217.00	2149.46 ± 763.05	OSHA PEL(Workplace)	5000 ppm (8-hour TWA)
	Winter	210	472.00	3284.00	2369.91 ± 828.13	OSHA PEL(Workplace)	5000 ppm (8-hour TWA)
CO (ppm)	Summer	210	3.48	13.42	8.69 ± 1.73	WHO air quality guideline (indoor air) OSHA PEL (Workplace)	10 mg/m³ (≈9 ppm) (8 h) 55 mg/m³ (≈50 ppm (8-hour TWA)
	Winter	210	4.18	23.74	15.63 ± 4.45	WHO air quality guideline (health-based) OSHA PEL (Workplace)	10 mg/m³ (≈9 ppm) (8 h) 55 mg/m³ (≈50 ppm (8-hour TWA)
$PM_{2.5}$ (µg/m³)	Summer	210	8.80	61.40	24.66 ± 11.24	WHO air quality guideline (health-based)	15 µg/m³ (24-hour mean)
	Winter	210	10.60	88.60	30.76 ± 18.49	WHO air quality guideline (health-based)	15 µg/m³ (24-hour mean)
PM_{10} (µg/m³)	Summer	210	28.60	224.80	96.94 ± 52.20	WHO air quality guideline (health-based)	45 µg/m³ (24-hour mean)
	Winter	210	32.40	408.60	131.29 ± 81.43	WHO air quality guideline (health-based)	45 µg/m³ (24-hour mean)
NH_3 (ppm)	Summer	210	1.09	63.47	21.83 ± 10.64	OSHA PEL (Workplace)	50 ppm (8-hour TWA)
	Winter	210	0.09	69.16	28.34 ± 14.82	OSHA PEL (Workplace)	50 ppm (8-hour TWA)

***Summer** = June and July; **winter** = November and December. **PEL**, permissible exposure limit; **TWA**, time-weighted average; **ppm**, parts per million (at 25 °C and 1 atm); **Pak-EPA**, Pakistan Environmental Protection Agency. Seasonal summaries were generated by combining data from 7 sampling sites within each farm. Minimum and maximum data are single data items and not means of the cells. Reference sources: WHO (2021), Pak-EPA (2010), and OSHA (n.d.; 29 CFR 1910.1000).*

Table 3: Exploratory repeated-measures ANOVA using five farms as subjects

Pollutant	Effect	df_1, df_2 (GG)	F	p (GG)	Partial η^2
NO_2	Site	2.65, 10.58	67.84	<0.001	0.944
	Month	2.03, 8.11	22.73	<0.001	0.850
	Site × Month	2.27, 9.09	9.48	0.005	0.703
	Day × Month × Site	26.292, 163.593	1.879	0.009	0.232
CO_2	Site	1.32, 5.29	2544.23	<0.001	0.998
	Month	1.12, 4.49	119.33	<0.001	0.968
	Site × Month	2.50, 10.01	42.83	<0.001	0.915
	Day × City × Month	17.404, 174.039	2.437	0.002	0.196
CO	Site	1.40, 5.59	52.75	<0.001	0.930
	Month	1.73, 6.92	189.28	<0.001	0.979
	Site × Month	2.15, 8.59	23.60	<0.001	0.855
	Day × Month × Site	30.964, 192.665	2.007	0.002	0.244
$PM_{2.5}$	Site	2.12, 8.48	311.98	<0.001	0.987
	Month	1.35, 5.40	70.36	<0.001	0.946
	Site × Month	2.73, 10.91	40.02	<0.001	0.909
	Day × Month × Site	27.211, 169.311	22.835	<0.001	0.786
PM_{10}	Site	2.04, 8.15	375.46	<0.001	0.989
	Month	1.24, 4.98	74.08	<0.001	0.949
	Site × Month	2.42, 9.67	10.63	0.003	0.727
	Day × Month × Site	27.357, 170.219	9.542	<0.001	0.605
	Day × City × Site	35.667, 156.043	0.292	1.000	0.063
NH_3	Site	1.45, 5.80	66.13	<0.001	0.943
	Month	1.06, 4.25	0.42	0.563	0.095
	Site × Month	1.47, 5.88	0.92	0.416	0.188
	Day × Month × Site	35.372, 220.095	1.255	0.166	0.168

*Each farm contributes 28 location-month means, each averaged across three readings. **GG** = Greenhouse-Geisser correction. The error term is farm × the corresponding within-farm effect. Partial η^2 is descriptive and may be unstable with five farms.*

The levels of NO_2 were more variable in the winter than in the summer, indicating seasonal variability in the distribution of NO_2. The primary model showed an important location × month interaction with an increase in NO_2 from the summer mean of 0.0271 to the winter mean of 0.0482 ppm. This is important since the winter increase does not need to be the same at each position. Heterogeneous patterns within-month are also suggested by the supplementary Day × Month × Site term (Table 3). The descriptive differences among selected farms, sampling locations, and months are illustrated in Figure 2.

3.2. CO_2 Concentrations at the Selected Poultry Farms

CO_2 concentrations varied significantly across measurement days in the City × Site, City × Month, and Month × Site models ($p < 0.001$), with significant temporal interactions across cities and months. Sampling site had a strong effect on CO_2 levels in the City × Site model ($F(6, 105) = 573.50$, $p < 0.001$, $\eta p^2 = 0.970$) and Month × Site model ($F(6, 112) = 5399.23$, $p < 0.001$, $\eta p^2 = 0.997$). The highest city means were recorded in Faisalabad on Day 1 (2274.71 ppm) and Lahore on Day 3 (2383.25 ppm).

The litter/manure area had the highest concentration on Day 1 (2980.40 ppm), while the Center of Shed recorded the highest on Day 3 (3122.40 ppm). Outdoor concentrations were lowest. December showed the highest monthly means on both Day 1 (2356.11 ppm) and Day 3 (2455.31 ppm), while June had the lowest monthly mean in the Month × Site model.

Most site comparisons were significant ($p < 0.001$), except between the Center of Shed and Litter/Manure Area ($p = 0.922$). CO_2 concentrations were higher in winter (2369.91 ± 828.13 ppm) than in summer (2149.46 ± 763.05 ppm). Relative humidity was positively correlated with CO_2 ($r = 0.328$, $p < 0.01$), whereas wind speed showed a negative correlation ($r = -0.162$, $p < 0.01$). Temperature was also negatively correlated, but not significantly ($r = -0.049$, $p = 0.315$). Figure 3 summarises the farm-level, sampling-location and monthly distributions of CO_2.

3.3. CO Concentrations at the Selected Poultry Farms

CO levels were significantly higher on Day 3 than on Day 1 in the City × Site, City × Month and Month × Site analyses ($p < 0.001$), with levels rising from 11.27 ± 4.73 to 12.90 ± 4.83, respectively. CO concentrations were significantly affected by the sampling sites, with the highest mean level in the Center of Shed (14.58) and the lowest in the Outdoor Site (8.10). Islamabad had the highest city mean (13.57), and Lahore had the lowest (10.34). The highest concentration was recorded during December (17.09), followed by November (14.17), July (9.35), and June (8.03). CO concentrations were higher and more variable in winter (15.63 ± 4.45).

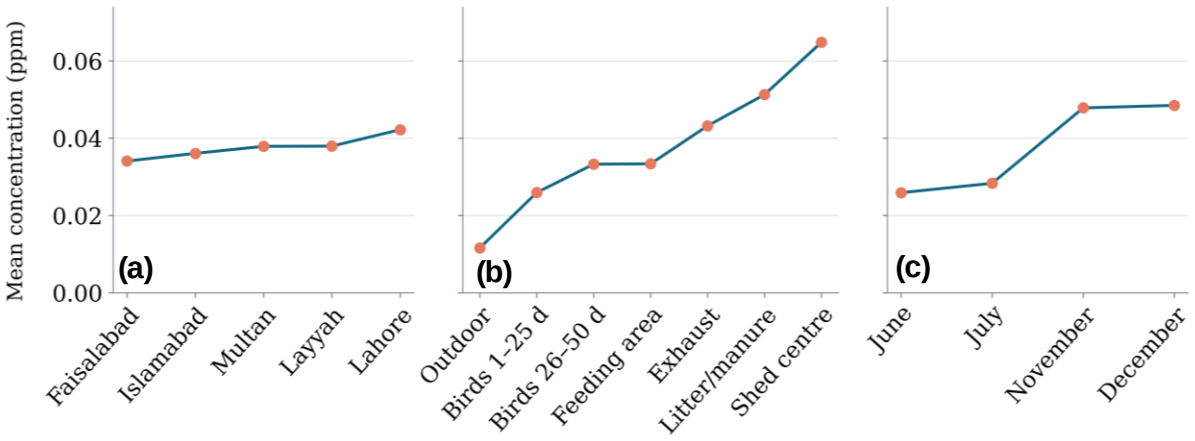

Fig. 2. Mean NO_2 concentrations by **(a)** selected farm, **(b)** sampling location and **(c)** month. Means summarise 84 readings per farm, 60 per location and 105 per month. A single sampled farm in each city is identified on the farm labels and bird age is represented in days.

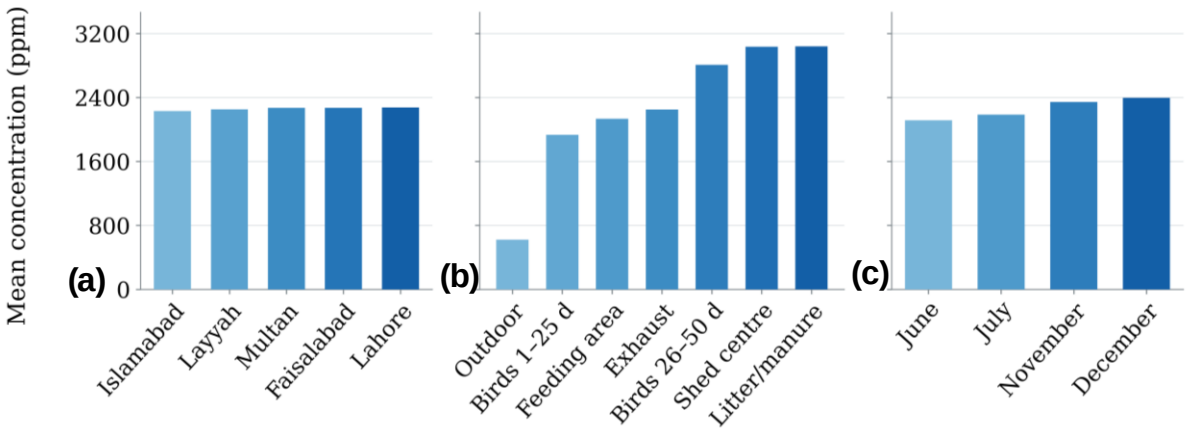

Fig. 3. Mean CO_2 concentrations by **(a)** selected farm, **(b)** sampling location and **(c)** month, expressed in ppm. Bars represent descriptive means.

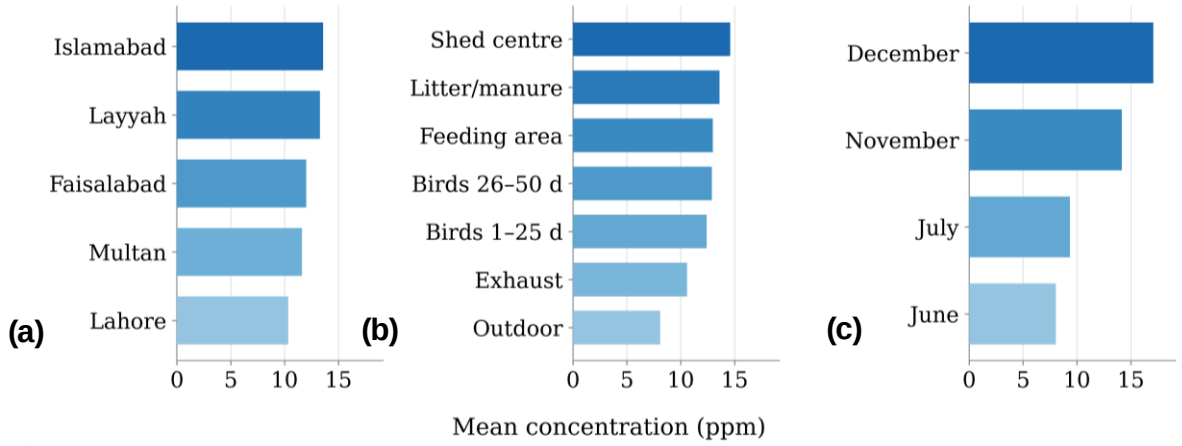

Fig. 4. Mean CO concentrations by **(a)** selected farm, **(b)** sampling location and **(c)** month, expressed in ppm. Horizontal bars represent descriptive mean values.

Relative humidity was positively correlated with CO ($r = 0.437$, $p < 0.01$), whereas temperature ($r = -0.361$, $p < 0.01$) and wind speed ($r = -0.509$, $p < 0.01$) showed significant negative correlations. The strongest correlation was found with wind speed. These between-farm, within-farm and monthly CO patterns are presented in Figure 4.

3.4. $PM_{2.5}$ and PM_{10} Concentrations at the Selected Poultry Farms

$PM_{2.5}$ concentrations varied significantly across measurement days in all interactions ($p < 0.001$), increasing from Day 1 (23.81 ± 14.32) to Day 2 (30.10 ± 17.53), followed by a slight decrease on Day 3 (29.23 ± 14.01). Significant Day × Site and Day × Month interactions were observed in the City × Site ($F = 9.20$, $p < 0.001$, $\eta p^2 = 0.345$) and City × Month analyses ($F = 2.87$, $p = 0.016$, $\eta p^2 = 0.067$), respectively. Temporal variation among months and sampling locations was also significant ($p < 0.001$) as seen in Month × Site, Day × Month and Day × Month × Site analysis. Sampling site significantly affected $PM_{2.5}$ concentrations ($F(6, 105) = 48.94$, $p < 0.001$, $\eta p^2 = 0.737$), with the highest mean in the Litter/Manure Area (47.78), followed by the Center of Shed (35.35). Concentrations were significantly higher in December than in July (mean difference = 12.30, $p = 0.001$), June (10.02, $p = 0.006$), and November (10.12, $p = 0.006$), but differences between the cities were not significant.

Similarly, PM_{10} concentrations differed significantly between the measurement days ($p < 0.001$), rising from Day 1 (93.03) to Day 2 (128.20) before declining on Day 3 (121.12). The City × Site interaction was significant ($F = 11.76$, $p < 0.001$, $\eta p^2 = 0.402$); Day × City and Day × City × Site interactions were non-significant ($p > 0.05$). Sampling site significantly influenced PM_{10} concentrations ($F(6, 105) = 59.58$, $p < 0.001$, $\eta p^2 = 0.773$), with the highest mean in the Litter/Manure Area (191.21), followed by the Center of Shed

(144.20). The City × Month interaction term was significant (F = 2.83, p = 0.018) in the City × Month model. The mean value in December was significantly higher than in July (52.00) and June (55.33), as well as in November (38.64). In addition, Month × Site, Month × Day, and Day × Month × Site interactions were significant (p < 0.001) for the Month × Site analysis, suggesting temporal and spatial differences in PM_{10} concentrations. The corresponding PM_{10} means by selected farm, month and sampling location are shown in Figure 7.

Relative humidity was significantly positively associated with PM_{10} (r = 0.408, p < 0.01) and $PM_{2.5}$ (r = 0.341, p < 0.01), while the temperature was significantly negatively correlated with them (r = -0.186 and -0.126, respectively, p < 0.01) and with wind speed (r = -0.277 and -0.267, respectively, p < 0.01).

$PM_{2.5}$ heat map provided more details than the pooled results, as the heat map revealed that the highest concentration was in the Litter/Manure Area in December on five selected farms, where the highest three-reading cell mean was recorded in Lahore at 79.73 µg/m³. The ratio of $PM_{10}/PM_{2.5}$ (4.11) suggested that coarse particles were predominant in the particulate matter measured. Location, month, and location × month terms were significant for both fractions (Table 3). The maximum values for individual readings for $PM_{2.5}$ and PM_{10} were 88.6 µg/m³ and 408.6 µg/m³, respectively. Figure 5 illustrates the $PM_{2.5}$ means across selected farms, sampling locations, and months.

Heat maps of $PM_{2.5}$ for both seasons were shown in Figure 6 (a,b). The position resolved $PM_{2.5}$ heatmap exhibits higher levels of $PM_{2.5}$ in December at all five selected farms, with the highest three-reading mean of the cell in Lahore (79.73 µg/m³). Heat maps of PM_{10} for both seasons were shown in Figure 8 (a,b). The corresponding PM_{10} heatmap shows the highest displayed winter litter/manure cell means in Faisalabad (351 µg/m³) and Multan (344 µg/m³), indicating marked variation across sampled positions.

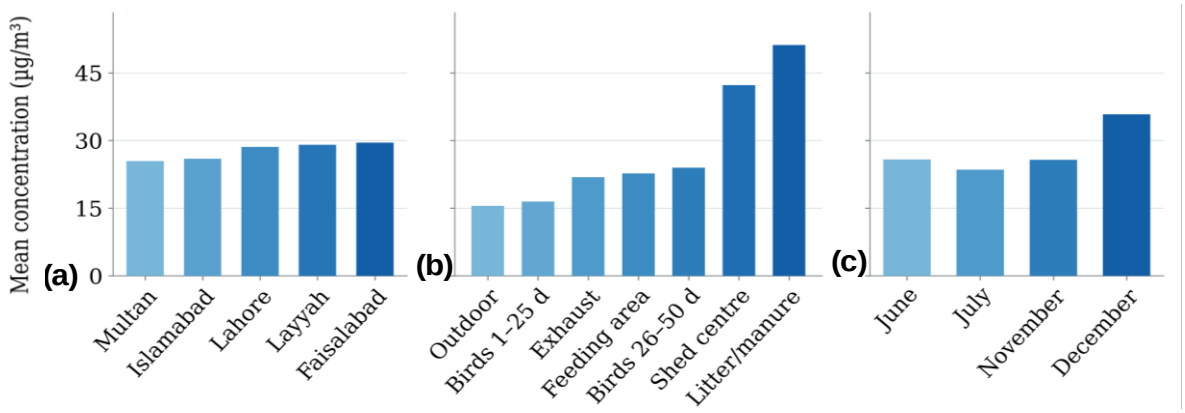

Fig. 5. Mean $PM_{2.5}$ concentrations by **(a)** selected farm, **(b)** sampling location and **(c)** month, expressed in µg/m³. Bars indicate the corresponding $PM_{2.5}$ mean values.

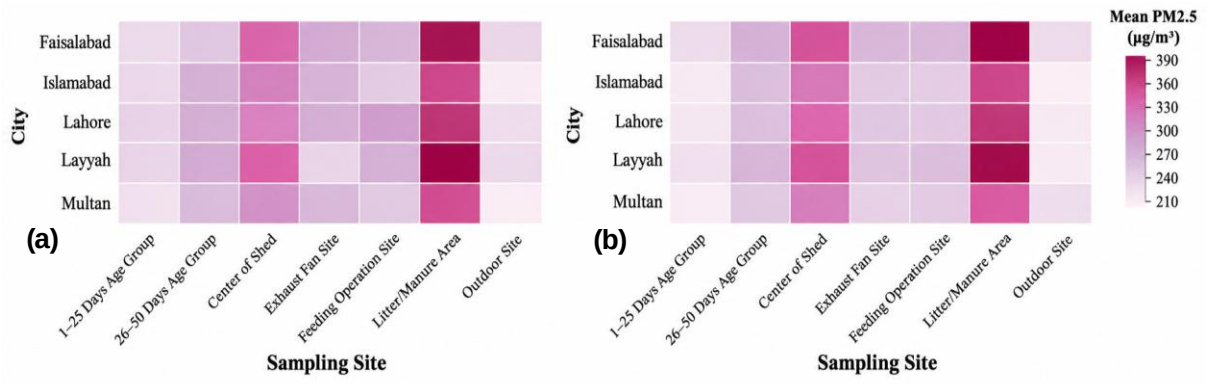

Fig. 6. $PM_{2.5}$ concentrations by sampled farm and monitoring position in **(a)** Summer and **(b)** Winter. Annotated cells are the means of three readings (µg/m³) from one farm per city.

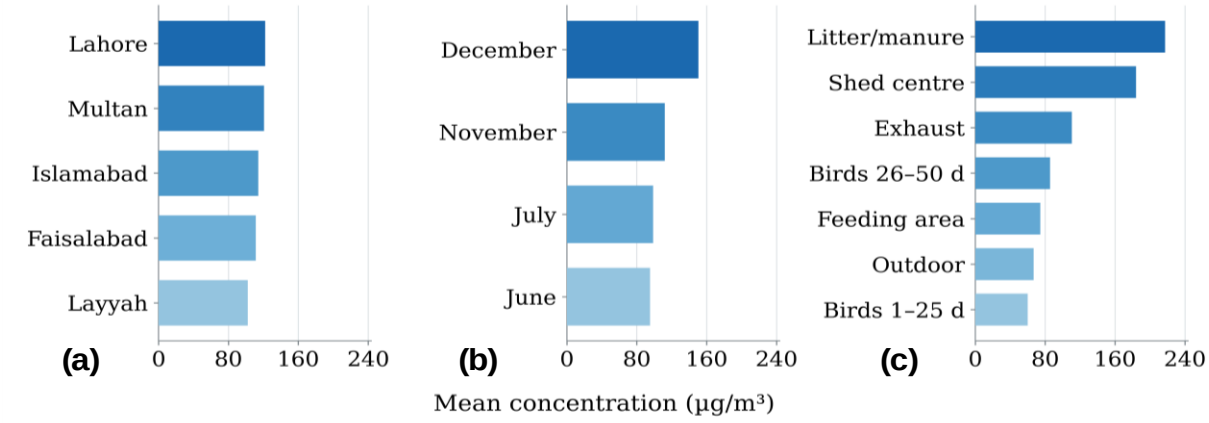

Fig. 7. Mean PM_{10} concentrations by **(a)** selected farm, **(b)** month, and **(c)** sampling location, expressed in µg/m³. Horizontal bars represent descriptive means.

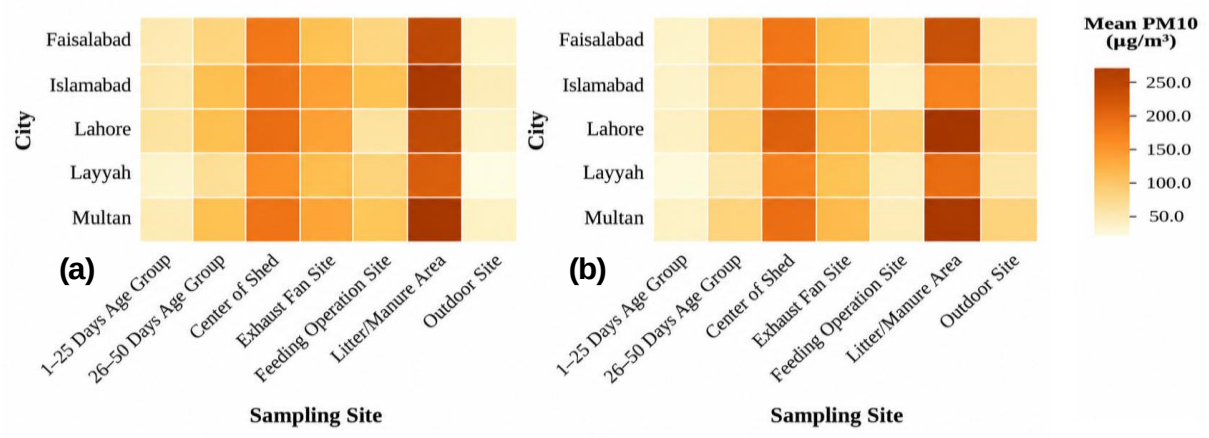

Fig. 8. PM_{10} concentrations by sampled farm and monitoring position in **(a)** Summer and **(b)** Winter. Annotated cells are the means of three readings (µg/m³) from one farm per city. A common colour scale is used across both seasons

3.5. NH_3 Concentrations at the Selected Poultry Farms

NH_3 concentrations did not differ significantly among the three measurement days, although means were slightly higher on Day 2 (25.36 ± 13.12) and Day 3 (25.16 ± 13.37) than on Day 1 (24.74 ± 13.48). The overall effect of Day was non-significant ($p > 0.05$); however, significant Day × Site interactions in the City × Site ($F(12, 210) = 124.60$, $p < 0.001$, $\eta p^2 = 0.877$) and Month × Site models ($F(12, 224) = 122.60$, $p < 0.001$, $\eta p^2 = 0.868$) indicated temporal variations among sampling locations. Significant differences were also observed among cities ($F(4, 105) = 3.97$, $p = 0.005$, $\eta p^2 = 0.131$) and sampling sites ($F(6, 105) = 18.88$, $p < 0.001$, $\eta p^2 = 0.519$). Among the selected cities, Layyah had the highest mean (27.75) and Multan had the lowest mean (22.42) for the city. The highest concentrations were found at the Center of Shed (32.98) and Exhaust Fan Site (31.55), while the lowest concentration was in the 26-50 Days Age Group (17.03).

Concentrations differed significantly between December and June in both monthly models ($p = 0.015$ and $p = 0.009$). The winter mean (25.95 ± 14.30) was slightly higher than the December mean (24.22 ± 12.17). The overall NH_3 concentration range was 0.09-69.16 ppm. There were no significant correlations between relative humidity, temperature, or wind speed ($p > 0.05$). The overall range was 0.09-69.16 ppm. Figure 9 presents the descriptive NH_3 patterns by selected farm, sampling location, and month. The summer-winter distributions across the six monitored pollutants and five selected farms are compared in Figure 10.

3.6. Microclimatic Parameters

Table 4 presents monthly microclimatic summaries. Temperature, Relative humidity, and air-speed summaries retain all 420 observations.

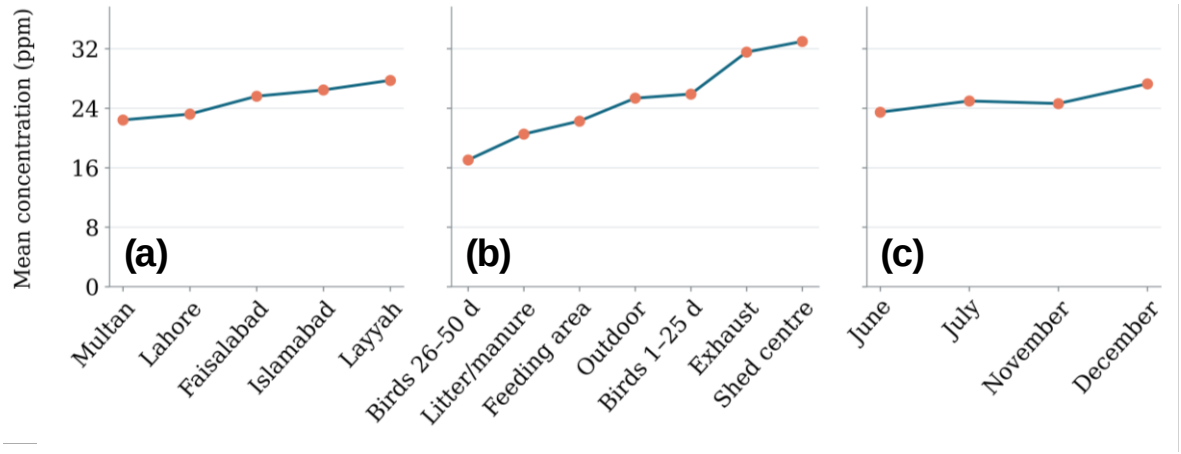

Fig. 9. Mean NH_3 concentrations by **(a)** selected farm, **(b)** sampling location, and **(c)** month, expressed in ppm. Lines connect categorical means.

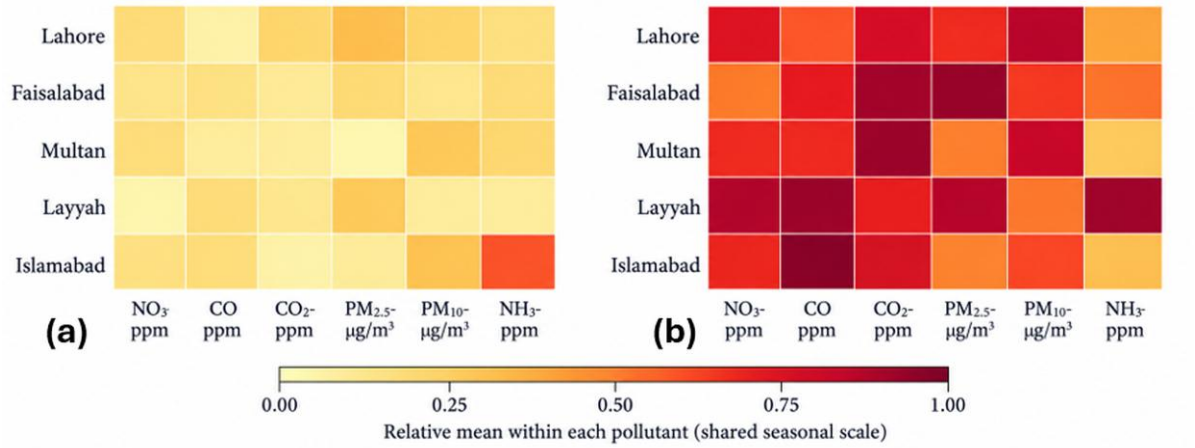

Fig. 10. Seasonal pollutant means at the five selected farms: **(a)** summer (June-July), left; **(b)** winter (November-December), right. Each cell summarises 42 readings. Annotations show mean concentration in the units beneath each column. Colour is normalised separately for each pollutant using the combined range of ten farm-season means, with the same scale in both panels. Colours do not represent health-risk categories or comparable concentrations across pollutants.

Table 4: Monthly microclimatic measurements

Month	N	Temperature (°C)	Relative humidity (%)	Wind speed (km/h)
June	105	37.35 ± 2.80	50.73 ± 6.38	13.71 ± 2.52
July	105	35.34 ± 2.64	63.19 ± 8.08	12.68 ± 2.62
November	105	24.28 ± 2.26	61.22 ± 4.40	9.48 ± 2.08
December	105	19.43 ± 1.98	66.16 ± 4.35	8.12 ± 1.93

Values are mean ± SD. Relative humidity, Wind speed, and temperature have n = 105 per month.

3.6.1. Temperature

There was no significant difference in the results obtained over the three days (p= 0.779). The interaction effects of Day x City or Site were also not significant (p > 0.05), which suggests that there was no significant difference between daily temperature patterns across locations. However, a significant difference in average temperature was noted between cities (p = 0.026). Differences between Islamabad and Multan, and Layyah and Lahore, were indicated by the Post hoc comparisons.

The City × Month interaction was non-significant (p = 0.455), and both City (p< 0.001) and Month (p < 0.001) were significant. Multan litter/manure site recorded the highest temperature (41.9°C), and Islamabad (outdoors) recorded the lowest temperature (15.4°C). The highest monthly mean temperatures were recorded in June (36.86), July (34.89), November (23.75), and December (24.27). In the Month × Site analysis, Month was

significant ($p < 0.001$), whereas Site ($p=.379$) and the Month × Site interaction ($p = 0.734$) were non-significant. The correlation results showed significant negative correlations with NO_2 ($r = -0.240$), CO ($r = -0.361$), $PM_{2.5}$ ($r = -0.126$), and PM_{10} ($r = -0.186$). The strongest negative correlation was observed with CO. However, temperature showed no significant correlation with CO_2 ($r = -0.049$, $p = 0.315$) or NH_3 ($r = 0.000$, $p = 0.995$).

3.6.2. Relative Humidity

In poultry houses, relative humidity increased significantly over the three days ($p < 0.001$), indicating a consistent upward trend. There was a significant difference between cities; Multan city had the highest average humidity value (67.04) for the litter/manure site, and Islamabad had the lowest (54.95).

The average humidity was the greatest in December (66.16) and the least in June (50.73) when compared across months. There was a large Day × City interaction, suggesting that the trend of changes in humidity over the 3 days varied between the cities. Similarly, the significant Day × Month interaction in the Month × Site model suggests that the daily pattern of humidity change varied across months. These results show that the relative humidity changed with time and in various geographical and seasonal situations.

All six pollutants correlation with Relative humidity were found to have significant positive correlation with CO_2 ($r = 0.328$), NO_2 ($r = 0.367$), CO ($r = 0.437$), $PM_{2.5}$ ($r = 0.341$) and PM_{10} ($r = 0.408$) at 0.01 significance level within the sampling locations. CO had the highest positive correlation, and PM_{10} had the second highest positive correlation. The correlation with NH_3 was weak, negative, and statistically insignificant ($r = -0.046$, $p = 0.345$).

3.6.3. Wind Speed

The results indicated that the wind speed was significantly different among the three days ($p < 0.001$), with the highest speed on Day 3 (12.15) and the lowest on Day 1 (9.77). There were no significant differences between the daily changes in the cities (Day × City interaction, $p = 0.542$).

The Day × Site interaction was significant ($p < 0.001$), indicating that the speed of the wind varied between sites. The interaction between Day, City, and Site was not significant ($p = 0.632$). A significant difference in average wind speed between the cities ($p<0.001$) and sites ($p<0.001$) was found. The mean wind speed for the overall study was the highest at Islamabad (approx. 12.65) and the lowest at Multan (approx. 8.83). The highest wind speed was observed at the Exhaust Fan Site from 13.30 on Day 1 to 15.61 on Day 3 across all the sites.

The City × Month interaction was non-significant ($p = 0.425$), but the City ($p < 0.001$) and Month ($p < 0.001$) were significant. June had the highest mean wind speed (14.91 on Day 3), followed by July (13.87), November (10.59), and December (9.22). Month and Site were significant ($p < 0.001$) but not interactive ($p = 1.000$) in the Month × Site analysis. Wind speed exhibited significant negative correlations with pollutants such as CO_2 ($r = -0.162$), NO_2 ($r = -0.252$), CO ($r = -0.509$), $PM_{2.5}$ ($r = -0.267$), and PM_{10} ($r = -0.277$). The strongest negative correlation was observed with CO. In contrast, wind speed showed a weak positive and statistically non-significant correlation with NH_3 ($r = 0.086$, $p = 0.077$).

Overall, relative humidity was positively associated with most measured air pollutants, while temperature and wind speed were generally negatively associated. The relationships indicate that meteorology may affect pollutant levels, but correlation does not imply causation.

3.7. Lead and Cadmium in Poultry Dust

The results showed that the levels of Pb and Cd were found to be variable in different cities, different months, and at different sampling sites. The highest mean concentration was found in the Center of Shed in Islamabad (1.147 ± 0.114), followed by Faisalabad (1.143 ± 0.156), while the lowest concentration was recorded from the Litter/Manure Area in Multan (0.303 ± 0.110).

Overall, the concentrations of Pb in general were higher in November and December than in June and July. Similarly, Cd concentration was found highest at the Litter/Manure Area at Lahore ($0.337 \pm$

0.096), followed by Layyah (0.321 ± 0.095) and Multan (0.306 ± 0.096), whereas the lowest concentration was recorded at the Center of Shed in Faisalabad during November (0.067 ± 0.010). Winter mean concentrations were higher than summer for both Pb (0.83 ± 0.31 vs. 0.62 ± 0.23) and Cd (0.23 ± 0.12 vs. 0.17 ± 0.08). Pooled farm means by sampling location and month are shown in Figure 11. Pearson correlation analysis showed a very weak positive relationship between lead (Pb) and cadmium (Cd) concentrations (Fig. 12) in dust samples ($r = 0.013$, $p = 0.924$, $N = 60$). However, this relationship was not statistically significant ($p > 0.05$). These results suggest that Pb and Cd concentrations were not significantly correlated in the collected dust samples.

3.8. Lead and Cadmium in Workers' Nails

Mean nail Pb concentration was 0.287 ± 0.060 μg/g⁻¹**)** in smokers (range: 0.217-0.368 μg/g⁻¹**)**) and 0.272 ± 0.060 μg/g⁻¹**)** in non-smokers (range: 0.169-0.324 μg/g⁻¹**)**) (Welch t(8.00) = 0.40, p = 0.700; Mann-Whitney U = 14, exact p = 0.841). Similarly, mean nail Cd concentration was 0.219 ± 0.062 μg/g⁻¹**)** in smokers (range: 0.134-0.287 μg/g⁻¹**)**) and 0.175 ± 0.030 μg/g⁻¹**)** in non-smokers (range: 0.135-0.216 μg/g⁻¹**)**) (Welch t(5.76) = 1.45, p = 0.200; Mann-Whitney U = 18, exact p = 0.310). Although the mean concentrations of both metals were descriptively higher in smokers, the differences were not statistically significant, and individual values overlapped between the two groups (Fig. 13).

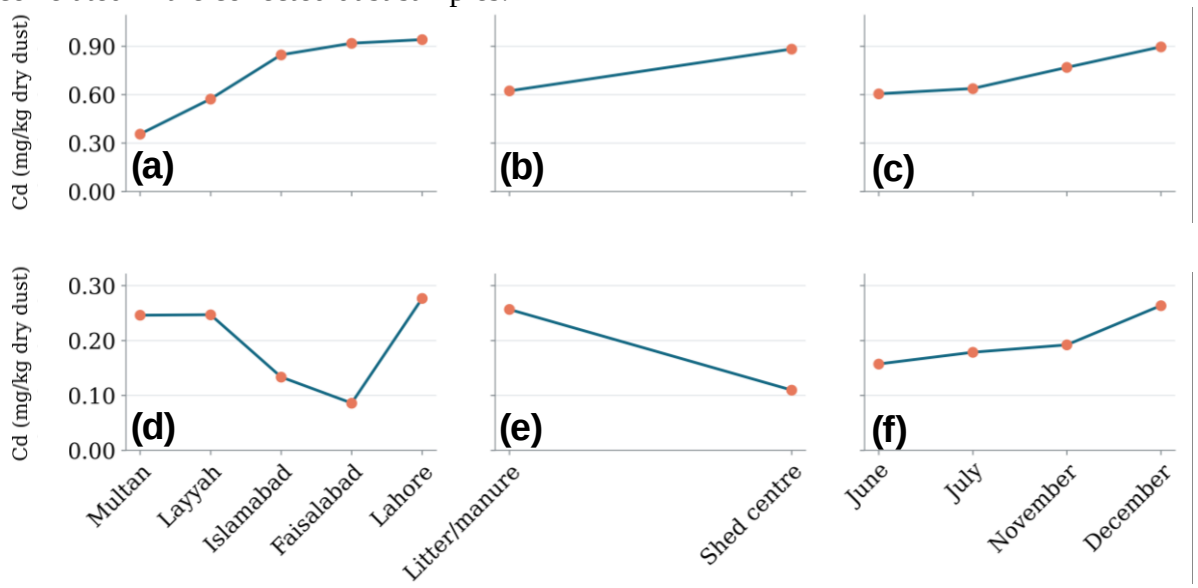

Fig. 11. Mean Pb **(a-c)** and Cd **(d-f)** concentrations in settled dust by selected farm, sampling location and month, expressed in mg/kg dry dust. Means summarise 12 samples per farm and 15 per month; location means comprise 24 shed-centre and 36 litter/manure samples. Shed-centre samples came only from Faisalabad and Islamabad, and litter/manure samples only from Lahore, Layyah and Multan.

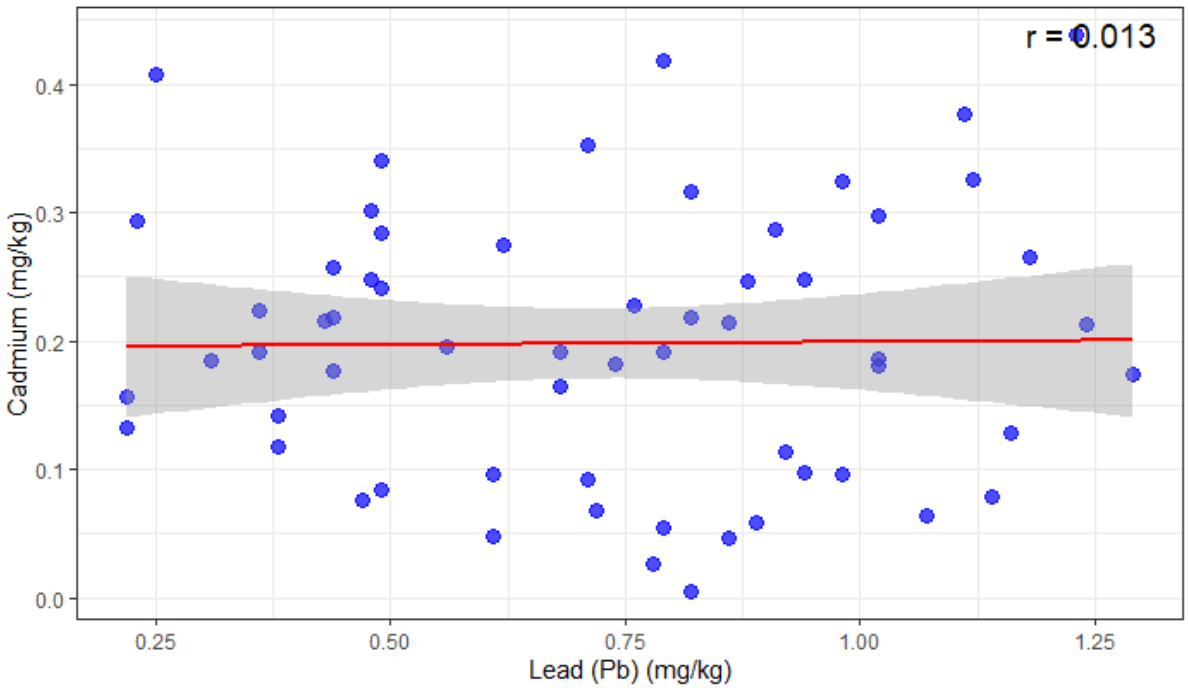

Fig. 12. Pearson correlation between lead (Pb) and cadmium in dust

Table 5: Descriptive nail-metal concentrations by smoking status

Metal	Group	N	Mean ± SD	Minimum-maximum (**µg/g⁻¹**)
Cd	Non-smoker	5	0.175 ± 0.030	0.135-0.216
Pb	Non-smoker	5	0.272 ± 0.060	0.169-0.324
Cd	Smoker	5	0.219 ± 0.062	0.134-0.287
Pb	Smoker	5	0.287 ± 0.060	0.217-0.368

Values summarise five workers per smoking group. SD denotes the sample standard deviation. Concentrations are descriptive.

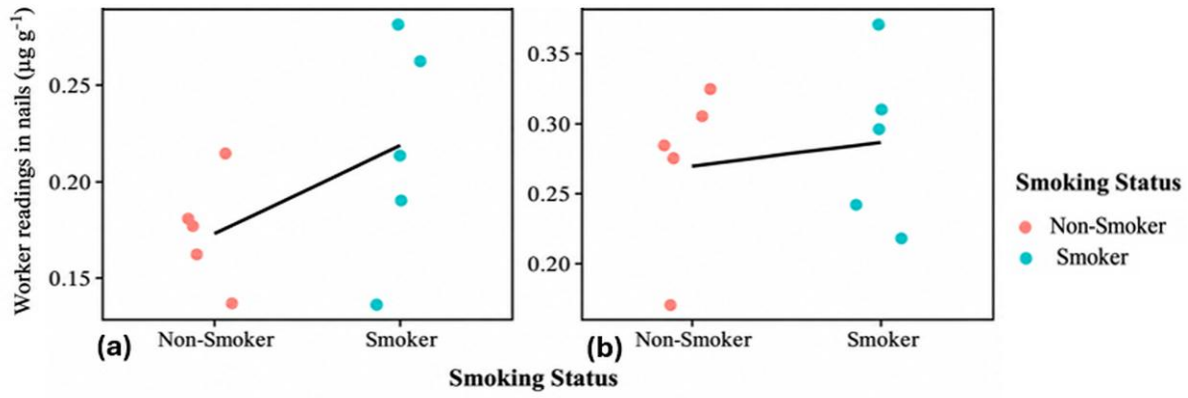

Fig. 13. Mean nail **(a)** Cd and **(b)** Pb concentrations (µg/g⁻¹) by smoking status. A similar number of workers contributed measurements for both metals.

3.9. Exploratory Self-Reported Symptoms among Poultry Workers

Table 6 shows the summary of the self-reported symptoms for the five smokers and the five non-smokers. Each group has one response at 20 percentage points. These are only intended to describe the patients and are not really accurate estimates of the risk of harm to health from smoking or occupations, because the numbers are too small.

Several symptoms were more prevalent among smokers, and wheezing was more prevalent among non-smokers. No clinical verification or pre-employment baseline or a matched non-poultry comparison group was available. The symptom table identified concerns for further investigation but could not establish smoking-related or occupational risk.

Table 6: Aggregate self-reported symptoms among the ten workers

Symptom	Smokers (n = 5)	Non-smokers (n = 5)
Headache	4 (80%)	2 (40%)
Cough	4 (80%)	2 (40%)
Skin irritation	3 (60%)	2 (40%)
Shortness of breath	4 (80%)	3 (60%)
Eye irritation	4 (80%)	3 (60%)
Chest tightness	4 (80%)	3 (60%)
Reported lung infection	4 (80%)	3 (60%)
Fatigue	5 (100%)	4 (80%)
Wheezing	2 (40%)	3 (60%)
Nasal irritation	3 (60%)	3 (60%)
Reported renal dysfunction	3 (60%)	3 (60%)
Reported cardiovascular disease	3 (60%)	2 (40%)

Values are n (%) within each group of five workers. Symptoms were self-reported and were not treated as clinically confirmed diagnoses.

4. Discussion

4.1. Spatial and Seasonal Patterns within the Poultry Houses and Occupational Relevance

The consistently higher NO_2 concentrations at the shed centre and litter/manure area, along with the lowest values outdoors, may be associated with differences in airflow and air exchange among sampling locations; however, these observations do not establish a causal relationship between ventilation conditions and pollutant concentrations. Abulude et al. (2025) reported a mean NO_2 concentration of 181.38 ± 16.48 ppb (0.181 ppm) in an indoor poultry house in Nigeria, attributing higher levels to combustion sources such as lanterns, stoves, and biomass burning. The concentrations were higher than those observed in the present study, indicating the role of indoor combustion sources and poor indoor air quality in contributing to NO_2. However, these spatial differences did not identify a common pollutant source. The study did not investigate the use of combustion appliances or other possible sources of emissions, so individual contributions could not be separated from background concentrations or variation in ventilation and air exchange.

Likewise, Nwanya (2025) found a negative correlation between NO_2 concentration and wind speed in poultry houses, and Mou et al. (2025) found that the increase in air movement over the litter caused a decrease in litter moisture in poultry houses. They also reported that sufficient ventilation and air movement are very important to enhance the quality of the indoor environment.

The findings did not establish respiratory injury among participating workers because no direct link was found between NO_2 exposure and self-reported health problems. Furthermore, the present study did not establish a causal relationship between NO_2 exposure and workers' health conditions. However, NO_2 is a reactive gas pollutant that can lead to oxidative stress and airway inflammation in the respiratory tract (Lu et al., 2023). Exposure to high NO_2 in the indoor environment has been linked to respiratory symptoms such as wheezing, cough, chest tightness, and asthma exacerbations, especially in susceptible people (Gao et al., 2026).

The concentrations were measured while workers were performing their routine tasks. The concentrations of NO_2 exceeded the WHO reference value of 0.013 ppm at several sampling locations during June, July, November, and December, whereas all recorded concentrations remained below the OSHA limit of 5 ppm. The measured concentrations were compared with the 24-hour air quality guidelines of the WHO only for comparison purposes, and the OSHA ceiling limits were used as reference concentrations for occupational exposure. These comparisons did not constitute comprehensive compliance monitoring. While the recorded NO_2 concentrations suggested potential exposure concerns for workers, the actual exposure and the sources of pollutants were not directly measured in this study, so a worker-level health risk assessment could not be completed.

The CO_2 concentrations were clearly different in the indoor activity areas and in the outdoor monitoring site. The CO_2 concentration was not significantly different between the Litter/Manure Area and the Center of Shed ($p = 0.922$), with a mean difference of -4.92 ppm. The main source of CO_2 is bird respiration, with a small part from litter. Dong et al. (2026) reported litter contribution of 9.0 % and 5.8 % in fast- and slow-growing broilers, respectively. Poor air

exchange also led to the accumulation of CO_2, as it was positively associated with relative humidity and had a significant negative correlation with wind speed ($r = -0.152$, $p = 0.002$). Additionally, Yang et al. (2026) found that CO_2 concentration dropped, and uniformity improved when ventilation was enhanced. The present measurements only distinguish concentration patterns, but not these contributions.

Winter heat conservation and changes in airflow may have contributed, but data on occupancy, fan duty cycles, and air-exchange rates were unavailable. Afroz et al. (2024) reported the CO_2 concentration in a commercial layer barn ranging from 1360 to 3300 ppm, and the factors influencing CO_2 concentration were found to be ventilation, chicken activity, temperature, and relative humidity. The present study indicated a higher CO_2 level in winter compared to summer because winter months were also colder, more humid, and less windy; the seasonal increase may be consistent with reduced ventilation and air exchange in cold weather. This seasonal difference was not explained, however, because rates of ventilation, fresh-air exchange, and fan operation were not directly monitored. Li et al. (2024) stated that there was spatial variation in the poultry house during winter for wind speed and ventilation. The CO_2 results showed a Day × City × Month interaction in Table 3. The CO_2 concentrations were between 463 and 3284 ppm and below the OSHA 5000 ppm 8-hour PEL. The CO_2 does not indicate adequate ventilation or health status but is applied in this study to determine if further testing of the IEQ is necessary. There was no direct measurement of the individual worker exposure. Therefore, the measured concentration cannot be considered a direct estimate of workers' personal exposure or used to establish associated health effects.

The mean CO concentration was higher in winter than in summer. The means at the shed centre were greater than outdoors, suggesting that local conditions in the shed centre were significant. A potential explanation is incomplete combustion from space heating, but no burner inventory, fuel record, on-site combustion measurement, or background-source assessment is provided in the study. Recent poultry-house studies have confirmed the importance of ventilation in controlling indoor air quality. Ghasemi et al. (2025) demonstrated that the type of ventilation plays a significant role in regulating the indoor airflow and microclimatic parameters in the layer house, and improper ventilation can cause accumulation of heat, humidity, and toxic gases. Similarly, Ma et al. (2022) found that ventilation is a major part of a poultry house and very important, particularly in winter, when combined with the removal of airflow and contaminants. However, ventilation rates were not measured, and other seasonal factors (e.g., heating, flock age at sampling, and litter condition) may also have contributed. The primary location × month interaction indicates fluctuation of CO concentration between months and locations. Temporal and spatial variation was also indicated by the reported supplementary Day × Site × Month term ($p = 0.002$; Table 3). The measured CO concentrations were above the WHO reference value of 9 ppm of 8-h in several sampling locations, whereas all recorded values remained below the OSHA exposure limit of 50 ppm 8-h exposure limit.

In the present study, the emission sources and personal exposure were not directly monitored, so the observed CO concentrations could not be directly attributed to specific sources or used to estimate workers' individual exposures. CO is a colourless, odourless toxic gas that forms carboxyhaemoglobin and therefore decreases the oxygen-carrying capacity of blood. Exposure to high concentrations may cause headache, dizziness, fatigue, and serious cardiovascular and neurological effects. NIOSH (2024) identified CO as a significant occupational hazard, particularly in enclosed or semi-enclosed workplaces. Similarly, Hamid et al. (2018) found that occupational air quality was a concern for workers at commercial poultry farms in Lahore and Sheikhupura. The results of this study indicated higher concentrations of CO at the Center of the shed and in winter. However, because CO emission sources and ventilation rates were not directly measured, the sources of elevated concentrations could not be determined.

The higher $PM_{2.5}$ concentrations observed in the Litter/Manure Area may be associated with the resuspension of fine materials during farm activities. However, other potential sources include background particles, feed handling, feathers, and animal-derived materials. Ahaduzzaman et al. (2021) pointed out that excreta, bedding, feed, and feathers are major components of poultry-house dust. This difference may also be affected by the particle fraction and monitoring location between Litter/Manure Area and outdoor concentrations. Fine particle size variations make it difficult to extrapolate near-litter/manure readings to worker personal exposure. Seo et al. (2024) found that PM_{10} and $PM_{2.5}$ concentrations increased during activity periods, as bird and worker activities disturbed feed and bedding, while decreased ventilation allowed particles to accumulate.

The higher PM levels measured during winter should be clearly distinguished from an increase in particle emissions. The changes in ventilation and air exchange may have influenced particle accumulation and removal during winter. An additional possible winter source of pollutants may be incomplete combustion in heating equipment. The results of Kim et al. (2025) also revealed that the amount of PM_{10} was higher in winter in a laying-hen house, while Wang et al. (2023) showed that the emissions of PM_{10} and $PM_{2.5}$ were higher in summer. These findings suggested that seasonal patterns may differ according to poultry-house type, ventilation, and manure-management conditions. The winter peak observed in the present study may therefore have reflected seasonal changes in indoor environmental conditions. However, ventilation rates were not measured directly.

In the present study, spatial effects were greater than city effects. Similarly, Yang et al. (2024) noted that $PM_{2.5}$ levels varied around a commercial laying-hen house in response to ventilation and meteorological conditions. The $PM_{10}/PM_{2.5}$ ratio (4.11) suggested that coarse particles predominated, potentially owing to the resuspension of litter, manure, and feed materials. In addition, Ni (2025) reported that hen activity also significantly increased PM_{10} production, pointing to the role of bird movement in particle production.

PM_{10} can irritate the respiratory tract, while $PM_{2.5}$ can penetrate deeper into the lungs and induce inflammation. In a study on broilers, Wang et al. (2026) found that exposure to $PM_{2.5}$ led to damage in the lungs, elevated inflammatory cytokines, and activation of the NF-κB pathway. Likewise, Matuka et al. (2023) mentioned the respiratory health risks among farm workers due to occupational exposure to poultry-house dust. The current study did not show a direct link between particulate matter levels measured and health responses from workers who participated in the study.

Higher NH_3 concentrations were observed at the Center of Shed compared with other sampling locations, which may be consistent with the decomposition of poultry excreta, whereas the low outdoor values are consistent with dilution. In the present study, however, the factors that might cause this spatial variation could not be identified because the sources of NH_3 emissions, the air exchange rate, and the ventilation were not measured directly. Barbosa et al. (2024) reported that higher ventilation levels decreased NH_3 concentrations in broiler houses, especially in the later stages of production. Similarly, Guo et al. (2025) highlighted the presence of NH_3 in the gas, particle, and litter phases of poultry houses, with its distribution influenced by farm management practices. Ahmadi Babadi et al. (2022) also reported that airflow patterns and ventilation influence NH_3 distribution, with higher levels in less ventilated areas.

At several sampling locations, NH_3 concentrations at several sampling locations recorded exceeding the OSHA permissible exposure limit of 25 ppm for 8-h, with the highest concentration observed in December. The present study did not directly evaluate individual workers' exposure. The significant Day × Site interactions, despite the non-significant overall Day effect, indicate that NH_3 concentrations changed over measurement days differently across sampling locations. Poultry workers may be exposed to ammonia together with dust, and combined exposure has been associated with a greater

reduction in pulmonary function than exposure to either contaminant alone.

4.2. Differences among Farms

Farm-level differences were significant for CO, NH_3 and $PM_{2.5}$ but were small relative to the within-farm (site) and seasonal effects. Because only one farm was sampled per city, these differences reflect characteristics of the individual farms such as ventilation type, bird capacity and litter material (Table 1) as much as, or more than, city-level climate or pollution, and they cannot be generalised to the poultry industry of each city.

4.3. Metal Concentrations in Dust and Nails

The buildup of feed leftovers, excreta, and bedding materials, which can retain metals, may be linked to higher Pb and Cd concentrations. Feed can contribute to metal accumulation in poultry-house environments, according to Yasmeen et al. (2023), who found that metal concentrations were generally highest in feed, followed by litter and air, with Pb being especially increased in litter. According to Ahaduzzaman et al. (2021), the main constituents of poultry-house dust include excreta, litter, feed, and feathers. Therefore, reducing metal accumulation may be aided by proper litter management, feed quality, and dust control. No national or international regulatory limits exist for Pb or Cd in poultry-house dust. The dust-metal results support the air monitoring results and show that some lead and cadmium were found in the dust as well. The average values of both elements from the descriptive analysis were found to be 0.622 mg/kg and 0.168 mg/kg for the summer and winter, respectively, while the averages of Cd were 0.832 mg/kg and 0.228 mg/kg for the summer and winter, respectively. However, the dust samples were collected from one type of sample point (center of shed), while litter/manure samples were collected from specific ones of the farm (Lahore, Layyah and Multan) from Faisalabad and Islamabad. This comparison of these two location classes would also be a comparison of isolated location effects and different farms and location management systems. The small sample size (five smokers and five non-smokers) limits the strength of the conclusions that can be drawn from these findings. Although mean nail Pb and Cd concentrations were descriptively higher among smokers that were consistent with tobacco smoke as an additional source of these metals, but the differences were not statistically significant. The results do not provide sufficient evidence to establish an association or causal relationship between smoking status and nail metal concentrations. Respiratory and general symptoms were found to be present in several workers in the sample, with no significant difference between the frequencies of the symptoms among smokers and non-smokers. The survey cannot confirm that symptoms were caused by the air pollutants from poultry houses, metals, or smoking, as it has not measured exposure at the individual level and no group of non-exposed people was included as a comparison group. The worker's findings should be considered preliminary and used to generate hypotheses.

4.4. Study Limitations

Only one purposively selected farm was included per city. In this context, therefore, farm identity was not separated from management and generalisation was limited to the overall context of the poultry industry.

Repeated measurements were taken to examine the conditions within each farm but could not substitute for farm replications. Thus, the results were not representative of all poultry farms. Monitoring was carried out during selected hours throughout four months; the monitoring period did not cover the full 24-hour standard limit. No direct worker exposure measurements were made. Moreover, activities of the heaters and farm operations were not directly monitored, and therefore, their potential influence on pollutant concentrations could not be determined. Location effects were not fully assessed because dust sampling sites were limited to individual farms. Limited interpretation of the health findings was also due to the small number of workers (n = 10) and the lack of a clinically assessed unexposed comparison group, as well as the absence of clinical diagnosis of reported symptoms. As such, the findings cannot be

used to make causal or broader population-level inferences.

5. Conclusion and Recommendations

Air-pollutant concentrations differed across sampling sites and months within the five selected poultry farms. NO_2, CO, CO_2, NH_3, $PM_{2.5}$ and PM_{10} varied substantially within poultry houses and across seasons, with the highest concentrations at the litter/manure area and shed centre and in winter, especially December. The differences between the farms were smaller and statistically significant only for CO, NH_3 and $PM_{2.5}$. Overall, NO_2, CO, $PM_{2.5}$ and PM_{10} exceeded their respective WHO guideline values, while NH_3 exceeded the OSHA permissible exposure limit at multiple sampling locations, indicating potential occupational air-quality concerns. Pb and Cd were detected in poultry-house dust and workers' nails; nail metal concentrations and several self-reported symptoms were descriptively higher among smokers, but these differences were not statistically significant in this small sample. The findings are preliminary and do not prove causal relationships between exposure and health outcomes. Within these limits, the results support improving natural and mechanical ventilation, especially in winter; managing manure and litter appropriately; controlling dust; ensuring PPE use; establishing smoke-free workplace practices; and periodically monitoring indoor pollutants and heavy metals. Regulatory authorities could promote periodic air-quality and occupational exposure monitoring, ventilation inspections, and worker training. Larger studies involving multiple and personal exposure monitoring are needed to provide a robust basis for occupational health policy in the poultry sector of Pakistan.

Acknowledgements

We would like to express our sincere gratitude to the Department of Environmental Science, Lahore College for Women University (LCWU), Lahore, Pakistan, for providing academic support and an encouraging research environment. We also acknowledge the support and cooperation of all those who contributed to the completion of this research.

Authors Contribution

All authors contributed to the conceptualisation and development of the research, participated in the research process and manuscript preparation, critically reviewed the manuscript, and approved the final version for publication.

Funding

This research was funded by the Higher Education Commission (HEC), Pakistan.

Conflict of Interest

The authors declare no conflicts of interest.

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