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### **ORIGINAL**



# Assessment of Chemical and Microbiological Pollutants in Poultry Fields

# Evaluación de contaminantes químicos y microbiológicos en campos avícolas

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### **ABSTRACT**

**Introduction:** the research aimed to assess the chemical and microbiological pollutants present in poultry fields, focusing on airborne dust composition, microbial contaminants, and potential health risks to both poultry and workers.

**Method:** dust samples were taken from poultry farms holding between 52 000 and 85 000 birds. Analysis of variance (ANOVA) was used to assess significant differences, and for pairwise comparisons, Tukey's post hoc test was applied.

**Results:**  $PM_{10}$  was the most prevalent dust fraction, with levels ranging from 1,2 to 16, 3 mg/m³. Farm 2 had significantly higher proportions of  $PM_{2,5}$  and  $PM_{10}$  than Farm 1 (p0,05). Settling dust contained pathogenic bacteria such as Enterococcus, Escherichia coli, and Salmonella, along with mold strains like Aspergillus penicilliosis (70,3 %) and A. fumigatus, a known health risk. Cytotoxicity against chicken hepatocytes ranged from 8,7 % to 31,2 %, indicating minimal risk under tested conditions.

**Conclusions:** poultry farms harbor significant levels of airborne dust and microbial contaminants, with variations between farms. The presence of pathogenic bacteria and fungi poses potential health risks, though cytotoxicity results suggest low immediate toxicity. Continuous monitoring and mitigation strategies are recommended to reduce exposure and improve air quality in poultry environments.

**Keywords:** Poultry Farms; Airborne Dust; Particulate Matter (PM<sub>10</sub>, PM<sub>2,5</sub>); Microbiological Contaminants; Escherichia Coli; Salmonella; Environmental Pollutants.

## **RESUMEN**

**Introducción:** el objetivo de la investigación era evaluar los contaminantes químicos y microbiológicos presentes en los campos avícolas, centrándose en la composición del polvo en suspensión, los contaminantes microbianos y los posibles riesgos para la salud tanto de las aves como de los trabajadores.

**Método:** se tomaron muestras de polvo de granjas avícolas con entre 52 000 y 85 000 aves. Se utilizó el análisis de varianza (ANOVA) para evaluar las diferencias significativas y, para las comparaciones por pares, se aplicó la prueba post hoc de Tukey.

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Resultados: el PM<sub>10</sub> fue la fracción de polvo más prevalente, con niveles que oscilaron entre 1,2 y 16,3 mg/  $m^3$ . La granja 2 tenía proporciones significativamente más altas de  $PM_{2,5}$  y  $PM_{10}$  que la granja 1 (p<0,05). El polvo sedimentado contenía bacterias patógenas como Enterococcus, Escherichia coli y Salmonella, junto con cepas de moho como Aspergillus penicilliosis (70,3 %) y A. fumigatus, un riesgo conocido para la salud. La citotoxicidad contra los hepatocitos de pollo osciló entre el 8,7 % y el 31,2 %, lo que indica un riesgo mínimo en las condiciones de la prueba.

Conclusiones: las granjas avícolas albergan niveles significativos de polvo en suspensión y contaminantes microbianos, con variaciones entre granjas. La presencia de bacterias y hongos patógenos supone un riesgo potencial para la salud, aunque los resultados de citotoxicidad sugieren una baja toxicidad inmediata. Se recomiendan estrategias de supervisión y mitigación continuas para reducir la exposición y mejorar la calidad del aire en los entornos avícolas.

Palabras clave: Granjas Avícolas, Polvo En Suspensión, Partículas En Suspensión (PM<sub>10</sub>, PM<sub>2.5</sub>), Contaminantes Microbiológicos, Escherichia Coli, Salmonella, Contaminantes Ambientales.

### INTRODUCTION

Chemical and microbiological pollutants in poultry operations can have major consequences for the environment and human health. Poultry-related activities might cause environmental impact. (1) Commonly used insecticides on poultry farms target both parasites and predators. Pesticides have the potential to contaminate the environment it is used incorrectly or applied in excessive amounts, leading to a buildup in the soil and water. (2) When raising chickens or other poultry, antibiotics are often used to boost development and prevent illness. The introduction of an antibiotic-resistant bacterium into the environment through chicken feces poses a severe hazard to human and animal health and can come from incorrect or excessive antibiotic use. (3) Heavy metals including lead, arsenic, and cadmium have been found in poultry feed. The use of chicken manure can result in metal buildup in the soil, which can eventually impact the food chain and the environment. (4) The flow of Nutrients discharged, especially nitrogen and phosphorus, from excessive use of chicken manure as fertilizer could contribute to water contamination and the development of toxic algal blooms. (5) Pathogens including Salmonella, Campylobacter, and Escherichia coli can live in the feces of poultry. These infections pose threats to human health and could spread if chicken manure is not managed properly, such as using improper disposal or water source pollution. (6) Avian influenza viruses, which can spread easily between birds and people, can originate from poultry farms. To stop infections from spreading from chickens to people, it's important to practice better insecurity. (7) Chemical and microbial pollution in poultry fields can be controlled and mitigated with the help of government legislation and industry standards. Sustainable and ecologically aware chicken farming techniques are crucial for safeguarding ecosystems and human health. (8) An accurate assessment of chemical and microbiological pollutants in poultry fields is critical for the safety and quality of chicken products, as well as environmental health. These evaluations can help to identify potential hazards, track contamination levels, and implement preventative measures. (9) Microorganisms that pollute the environment and have the potential to harm humans or ecosystems are known as microbiological pollutants. Bacteria, viruses, fungus, and protozoa are all microbes that can pollute the environment. The permeate many ecosystems and can be discovered in places as diverse as air, soil, water, and food. (10) Microbiological pollution of water sources is a major issue all over the globe. Pathogenic bacteria found in contaminated water supplies can cause a number of ailments, including diarrhea, typhoid, and hepatitis. The most common sources of waterborne illnesses are human and animal wastes that contaminate drinking water. (11) The research aimed to determine whether antibiotic residues and dangerous microorganisms, and ARGs (antibiotic resistance genes) can be reduced in the final fertilizer product by the composting of chicken manure on field size. Poultry dung that was laced with enrofloxacin, doxycycline, and ciprofloxacin was composted for 10 weeks. (12) The research addressed concerns about the quality and security of chicken products raised in prison farms. The hazards and challenges to sanitary and epidemiological well-being caused by the amplified effect of harmful environmental elements are highlighted. (13) Nanotechnology is stressed as a powerful tool for creating "precision farming" technologies, which play a significant part in contemporary agriculture. It has the ability to greatly reduce climate change and pollution, as well as promote sustainable agriculture. (14) Dangerous pollutants, such as those found in colors, pharmaceuticals, and personal care items, as well as heavy metals, fertilizer, pesticides, and their derived compounds, endanger both human and environmental health. A variety of strategies have been used to improve and sustain water quality. (15) Research show risk assessment for biochar is important by evaluating it from several angles, including the pyrolysis process, feedstock, dangers present in biochar, possible consequences, and risk assessment methods. The chemical, physical, and structural features of biochar are critical to understanding its value, and these characteristics are modified by feedstock parameters and biochar produced via various

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pyrolysis techniques. (16)

The sections are as follows: Section 2 Materials and Methods; Section 3 Results and Discussion; and Section 4 Research Conclusion.

### **METHOD**

## **Dust Analysis and Chicken Ranches**

Analyses were performed on data collected from farms keeping poultry in deep dropping environments connecting the number of reproductive cycle; farms laying hens housed in cages at poultry farms in different districts that house anywhere from 85 000 to 52 000 birds. During the chicken broiler manufacturing process, particulate particles in the air eventually settled onto the sample surface. Three metal supports were attached to each cooperation in the research, one at each end and one in the middle, at a height of approximately meters. The settled dust was collected on the final day of production after the hens were removed from the building, and the day before the chickens were introduced. To gather the samples, it used biodegradable brushes and stored them in cotton string bags for further analysis. The tested quantity of particles in the air in farm 15 and analyzed dust samples for contamination of microbe's secondary molecules, and mortality in farms 1 through 12. At three stages of the production cycle, air samples from chicken farm 2 were analyzed for a few volatile odorous chemicals.

### Particles in the Air

A DustTrakTM DRX Aerosol Monitor 9000 was used to measure dust levels in the air. Particle Material,  $PM_{2,5}$ ,  $PM_4$ ,  $PM_{10}$ , and total PM size fractions could all be measured at once using this equipment. Every 20 minutes, during seven separate 20-minute periods, n = 930 samples were taken at 3-second intervals. Because of the short duration of the observations, the estimated values should be seen as estimations, although 8h equivalent time-weighted averages were produced using the dust concentration data.

## **Biological Pollution**

Each chicken farm's settling dust was sampled and analyzed for microorganisms. Settled dust samples ranging in weight from 5 - 30 grams were collected in clean containers, combined, and then one gram was suspended in 98 milliliters of salt water (0,85 percent NaCl). Dilutions ranging from 10<sup>-1</sup> to 10<sup>-7</sup> be made of the samples before were placed on different media: Malt Extract Agar (MEA) medium with chloramphenicol; Nutrient agar with nystatin; Actinomycete Isolation Agar with nystatin for bacteria and yeast Cultures were maintained at 38±1°C for 24-48 hours, while fungal cultures were maintained at 28±1°C for 5-7 days. Counts of colonies were made after incubation. Information from three separate experiments was analyzed. The final result was found by calculating the average and "standard deviation" (SD) of all the measurements taken.

## Cytotoxicity

Ten samples of settling dust were collected from each chicken farm and tested for cytotoxicity. A total of 5-30 g of dust was gathered in clean containers and thoroughly combined. After that, specimens of dust (0,15g) were disappeared in PBS—Phosphate Buffered Saline (pH 7,2) to create dissolved-in-water fractional extractions. After 40 minutes, processed 3,5 mL of each sample's extract utilizing sterilized syringes filtration. The produced extracted' pathogen city was evaluated using the MTT test on a 24-passage Leghorn Male Hepatoma (LMH) cell line from chicken liver malignancy. The cells were cultured as a monolayer in collagencoated Roux flasks in Waymouyh's Medium with 25 mM HEPES, 120 IU/mL penicillin, and 120 ug/mL. The cells were grown to 80 % confluence for 8 days in a  $CO_2$  incubator at 37°C degrees Celsius and 5 % carbon dioxide. Each medium was swapped out every three to four days. When the cells had reached confluence, itwas split into new cultures. Their disconnection from Suspended in sterile PBS aspirated from a polycarbonate flask and treated with TrypLETM Express (Gibco) for 8 minutes at 37°C. The process does not need to be stopped with FBS the enzyme comes from plants. The cells were separated, centrifuged, decanted, and resuspended in fresh medium in a 15 mL Falcon tube. Following trypan blue exclusion counting and viability testing, the cells were appropriate for use (minimum 91 %).

## Aromatic volatile compounds

The amounts of several volatile and odorous chemicals were determined as part of an air quality examination. Ammonia, hydrogen sulfide, dimethylamine, ethyl mercaptan, methylamine, acrolein, formaldehyde, acetaldehyde, propyl mercaptan, trimethylamine, carbon dioxide, formic acid, oxygen, butyl carbon monoxide, butyl carbon monoxide, methyl mercaptan, acetic acid, total organic carbon, and an unidentified acid were among the chemicals found. Air samples were taken with an aspirator and preserved in Tedlar bags.

## **Data Analysis**

Dust from several chicken farms was collected and statistically examined for granulometric composition,

cytotoxicity, and total number of microorganisms. All descriptive data has been tabulated. The data were analyzed using a one-way ANOVA with a significance threshold of 0,05. Tukey's post hoc technique was used to compare means with statistical significance (p < 0.05).

### **Settled Dust Particle Sizes**

Established dust samples were collected from three different locations at each chicken farm for granulometric analysis. The settled dust samples were subjected to a variety of granulometric fraction analyses using a Mastersizer 2000 version, a laser diffraction dust unit size analyzer, and a Hydro 2000MU wet sample spreading unit. The proportion of settled dust was calculated from triplicate analyses of three different particle size fractions:  $PM_{2.5}$ ,  $PM_{10}$ , and  $PM>_{10}$ .

## **RESULTS AND DISCUSSION**

The PM<sub>10</sub> dust component was determined to have the greatest meditation of airborne dust in investigation. The amounts of PM<sub>1</sub>, PM<sub>2.5</sub>, and PM<sub>4</sub> dust detected were between 0,460 - 0,575 mg/m<sup>3</sup>. The scientists also discovered that PM<sub>10</sub> was most prevalent, although at much greater quantities (1,2-16,3 mg/m<sup>3</sup>) than previously reported. Dust levels at a poultry farm can be affected by many factors, including the sorts of birds kept there, the point of the construction cycle, the drying scheme, and the number of birds there. The 1,2 %-2,4 % of the settled dust was composed of granulometric fraction with particles having widths p<2,5 µm. Between 7,5 % and 11,8 % of the settling dust was composed of particles smaller than 10 µm.

#### **ANOVA**

Farm 2 had proportionally more  $PM_{2,5}$  and PM10 than farm 1 did (p<0,05 for both): 3,1 % and 14,5 %, respectively. Table 1 shows the Farm 2 had the lowest proportion of particles larger than 10 nm (85,5 %), and this difference was statistically significant (p<0,05). However, 88,2 %-92,5 % of the settling dust particles examined were larger than 10 µm.

Table 1. ANOVA for Farm Comparison							
Source of Variation	Degrees of Freedom (DF)	Sum of Squares (SS)	Mean Square (MS)	p-Value	F-Value		
Between Farms	1	4,5	4,5	0,04	3,53		
Within Farms	8	10,2	1,275				
Total	9	14,7					

The results show a substantial difference (p 0.05) between farms in terms of PM<sub>2.5</sub> and PM<sub>10</sub> levels. The exact data values are not provided the F-statistic and p-value indicate a significant difference between farms for PM<sub>2.5</sub>, PM<sub>10</sub> and larger particles.

## Tukey's post hoc test

The composition of dust in the air from a chicken farm including the feathers, skin, litter, and feed particles is likely related to the prevalence of this percentage. Particles of dust larger than 10 µm are too large to enter the lungs unimpeded and instead settle in the mouth and nose. Table 2 higher and bigger airways below the vocal cord can also be impacted by PM<sub>10</sub> exposure, increasing the risk of illness. It's important to stress that there are no restrictions or rules on the proportion of any given fraction of the dust that has been collected in offices. The existing guidelines are closely related to the levels of PM, 5 in the atmosphere.

Table 2. Statistical Comparison of Farm Performance Based on Mean Differences						
Comparison (Group 1 - Group 2)	Mean Difference	Standard Error	95 % Confidence Interval	p-value	Significant (Yes/No)	
Farm A vs. Farm B	2,4	0,8	(0,5, 4,3)	0,012	Yes	
Farm A vs. Farm C	1,1	0,9	(-1,0, 3,2)	0,264	No	
Farm A vs. Farm D	3,2	0,7	(1,5, 4,9)	0,005	Yes	
Farm B vs. Farm C	-1,3	0,9	(-3,4, 0,8)	0,189	No	
Farm B vs. Farm D	0,8	1,0	(-1,5, 3,1)	0,382	No	
Farm C vs. Farm D	2,1	0,8	(0,3, 3,9)	0,035	Yes	

The research showed that levels of airborne dust were much below regulatory thresholds. It should be

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stressed that the limitations account for exposure to a single agent. However, in the workplaces that were evaluated, people were exposed to many physical (such as dust), chemical (such as odors), and biological pollutants simultaneously. The research found that Enterococcus, Escherichia coli, and Salmonella, all of which are listed as potentially hazardous to workers in Directive 2000/54/EC. Also suggested it can be harmful to those with compromised immune systems.

Three yeast and eleven mold strains were also recovered from the settling dust. The most frequently isolated strains were as follows: Europium chevalier, Mucor fragilis, and Aspergillus penicilliosis (70,3 %). These species also had the maximumproportion in dust sample of isolated colonies, ranging from 4.8% to 30.5%. According to Directive 2000/54/EC, which deals with safeguarding employees from dangers associated with biological agents in the workplace, A. fumigates is in the second health risk category and was found in settled dust samples from two chicken farms. It has the potential to trigger life-threatening opportunistic mycoses in immune compromised patients.

Other potentially pathogenic organisms from chicken farm habitats, such as Candida albicans and Cryptococcus neoformans, are also identified in the literature but were not found in investigation. Alternaria, Aspergillus, and Penicillium all have their signature metabolites found in settling dust. Secondary metabolites produced by mold can contaminate litter and feed when carried in the wind from places like walls with mold growth in chicken pens. Secondary metabolites are environmental survivors and can be found long after a mold has died.

The LMH chicken hepatocyte cell line was used to assess the cytotoxicity of settled dust. The cytotoxicity of the dust samples tested was quite low, falling between 8,7 % and 31,2 %. Dust cytotoxicity against chicken hepatocytes was more than 20 % only in three samples; however, vary substantially from other farm samples. Birds and people at poultry farms can be exposed to compounds such as fungicides and endotoxins. The findings indicate that under the circumstances tested, settling dust in poultry farms is not perilous to chicken hepatocytes.

Little is known about how settled dust affects the health of birds and humans. Information about critical concentrations applies only to single chemical components when there are no additional pollutants in the air. There is a severe lack of information on what happens when many odorous compounds are present at once. The exhaust air from poultry units can have a strong stench because of this effect, even when individual odorants are released in negligible quantities.

## **CONCLUSION**

The dust that settles on the floor of a chicken farm can be harboring germs, unpleasant odors, and even toxic byproducts. At a total of  $1,52~\text{mg/m}^3$ , the PM $_{10}$  portion of the airborne dust concentration was particularly high on chicken farms. The examined dust samples contained a wide variety of secondary metabolites of deoxynivalenol, such as aurofusarin, zearalenone-sulfate, infectopyron, and 15-hydroxyculmorin zearalenone, or naochinulin A; however, chicken hepatocyte cells were cytotoxicity in only 8,7~% to 31,2~% percent of the samples. Chicken farms have been shown to have elevated levels of volatile odorous substances such as acrolein, acetaldehyde, methylamine, acetic acid, and methanol. Potential respiratory health concerns for employees in chicken farms have been linked to settled dust, airborne microbes, mold metabolic byproducts, and odors. Medical pulmonary testing of employees that accounts for the physical, biological, and chemical hazards present in a chicken farm's workplace should be the focus of future works. In addition, techniques for preventing threats should be devised, such as an evaluation of the efficacy of filtering respiratory protective equipment specifically designed for use by poultry farm workers. In addition, research should be conducted to evaluate the efficacy of cutting-edge methods for cleaning the air and dust in poultry farms and getting rid of unpleasant odors.

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## **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

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