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Research article

Laying Hens Feathers as a Bio-indicator for Monitoring Heavy Metals (Pb, Ni, and Cd) Pollution in Industrialized Regions of Wasit Governorate, Iraq

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ABSTRACT

The widespread and increasing industrial and anthropogenic activities bring an enormous pollution risk of heavy elements into the environment, which has negative consequences for both the environment and human health. As a result, the bioaccumulative and bioarchival nature of the keratin matrix of the feather makes these structures excellent non-destructive bioindicators for the detection of environmental contamination, especially from laying hens. In the present study, the samples from the chickens named Feather (breast and wings) were collected a total of 30 hens across the five poultry farms of different anthropogenic influence levels, such as the pristine one as a control and four other sites with different anthropogenic impacts due to petroleum emissions, thermal power plant effluents, brick kiln particulates, and highway traffic exhaust. The samples were analyzed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) with two different subsample types: washed and unwashed, to distinguish between external deposition and internal bioaccumulation. The results indicated that the general tendency of heavy metal concentrations in both washed and unwashed feathers, as mean concentrations across all sites, was $Pb > Ni > Cd$. This order was, however different at different sites, with Ni concentrations exceeding Pb at Sites 1-3. The highest contamination by Pb was found in unwashed feathers at site 5 (6.00 mg/kg dry weight, mainly due to vehicular emissions and fossil fuel combustion) and site 3 (4.60 mg/kg dry weight, mainly due to fossil fuel combustion). In contrast, Site 3 accumulated the highest levels of Ni (5.40 mg/kg) and Cd (0.50 mg/kg). The statistical analysis showed highly significant differences between the sites ($P < 0.001$). In addition, washed and unwashed feathers showed a high degree of correlation for both Pb ($r = 0.812$) and Ni ($r = 0.845$). The results of the study demonstrate that laying hen feathers are an effective, sensitive, and reliable bioindicator of elemental deposition in the atmosphere and local ecosystem health.

1. Introduction

In recent years, concerns have been growing regarding toxic metals and their impact on human, animal and environmental health (Iqbal *et al.*, 2023). This is due to the pollution produced by increased industrial, commercial, mining and agricultural activity (Das *et al.*, 2023). Heavy metals are naturally occurring with minimal levels in the environment. They are continuously released into the environment from the natural processes such as volcanic activity and rock weathering (Masindi

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et al., 2021). In addition, industrial processes and some agricultural activities have greatly contributed to the addition of different metal species to freshwater environments (Tchounwou *et al.*, 2012). Therefore, the heavy metal polluted status has become an important matter of concern in recent years (Timothy and Williams, 2019). These metals are classified as non-biodegradable and persistent metals. They are able to bioaccumulate in living things. A certain amount of exposure to these metals may be detrimental to many organisms (Smart and Hodgson, 2018). The primary sources of heavy metal emissions to the environment are industrial emissions, organic emissions, vehicle exhaust gases, garbage combustion and energy generation (Nardiello *et al.*, 2019).

Biomonitoring is the study of bio-species or ecological communities which can be observed to change as a result of changes in the natural environment. Biomonitoring studies provide a more holistic view of the effects of human activities and contaminants on the health of particular species or biological communities, compared to laboratory studies that test toxicological effects. Birds have been known to be very sensitive to changes in the environment (Jones *et al.*, 2023) and they are commonly used to represent habitat quality, pollution, biodiversity loss and potential human diseases (Izah *et al.*, 2023). Heavy metals that have been absorbed by the birds from food, water and other environments are concentrated in their tissue and organs. In addition, it is known that birds deposit heavy metals in their tissues, eggs, egg shells, feces and feathers in a way to eliminate them from the body (Durmuş *et al.*, 2023).

Feathers of birds from different taxa have been used in numerous ecotoxicological studies (Kushwaha, 2016; Zarrintab *et al.*, 2016; Yamac *et al.*, 2016). As a result the following are very important for sampling and analysis of feathers: i) they tend to accumulate certain metals (As) that other body organs do not; ii) they play an important role in the excretion of heavy metals (Hg, As, etc); iii) they are easily sampled (live, dead, or museum samples); and iv) they provide an opportunity to analyze endangered species (Celik *et al.*, 2021). Bird feathers were used to assess bioaccumulation of elements in the atmosphere of different environments and it was found that the concentration of these elements in the feathers correlated with the concentration of them in the atmosphere (Hoff Brait and Antoniosi Filho, 2011). Air dust, which was detected with high levels of heavy elements by monitoring using an air dust device (Al-Shammari, 2020) is the most common pollutant in Wasit.

Avian feathers, particularly of laying hens, are good non-invasive bioindicators for monitoring heavy metal contamination such as lead (Pb), cadmium (Cd) and nickel (Ni). Jaspers *et al.* (2004) performed a basic methodological assessment of the relative importance of exogenous deposition and endogenous accumulation in bird feathers. They demonstrated that older feathers and longer environmental exposure led to significantly higher levels of heavy metals like lead and cadmium, showing that a large part of the metal response in feathers is due to surface accumulation and not only internal somatic burden. Abduljaleel *et al.* (2012) studied the distribution of metals in different tissues of birds and measured the concentrations of heavy metals in various tissues of chickens and quail from poultry farms in Selangor, Malaysia. Their results showed that among the internal organs, feathers had the highest bioaccumulation potential for lead (Pb) and copper (Cu). The authors attributed this higher accumulation to a twofold mechanism, namely endogenous uptake through blood circulation during feather development and exogenous deposition of particulate matter on the feather keratin matrix. However, Lodenius and Solonen (2013) have noted that the distinction between external contamination and internal uptake in tissues needs to be dealt with at a methodological level through stringent laboratory washing protocols. True dietary and physiological

bioaccumulation can only be accurately measured when the surface deposits are completely removed, which can be done by sequential rinsing of the sample with organic solvents (e.g., ethanol or acetone) and deionized water prior to acid digestion. Recently, Yao *et al.* (2021) investigated the accumulation of lead and cadmium in laying hens in different environments. Their research supported the use of feathers as a proxy for long-term environmental exposure through surface deposition and slow internal bioaccumulation in the kidneys and liver, relative to known thresholds in the muscle and visceral tissues. They concluded that chicken feathers are very sensitive diagnostic tools for detecting heavy metals such as lead, cadmium and nickel, provided that exogenous surface contamination is properly isolated during the preparation of the sample.

Despite the importance of biomonitoring systems and their effectiveness in monitoring pollutants in the environment, there are very few local studies, with the majority focusing on the use of plants. Therefore, this study was conducted to quantify the concentration of certain heavy elements in the feathers of laying hens in five different areas from Wasit Governorate and comparing the washed feathers with the unwashed feathers of laying hens in the five areas, to evaluate the efficiency of using the feathers in environmental monitoring. Bird's feathers have a potential in environmental studies to assess ecological health of the local ecosystem, and can be used as a non-destructive bio-monitoring tool.

2. Materials and Methods

2.1 Study area

The samples were collected from five fields of laying hens that were distributed in 5 different locations in the Wasit Governorate to represent different levels of environmental exposure, one field being located in a remote agricultural area with no anthropogenic influence and the other four being located in areas that are close to specific anthropogenic activities. Site 2 is in the Al-Ahrar district (9.5 km south from the Al-Ahdab oil field) and is impacted by petroleum emissions. Site 3, in the Al-Zubaidiya region (8.5 km south of Al-Zubaidiya Thermal Power Plant), is exposed to effluents from the power generation. Industrial particulate matter affects Site 4 in the Al-Hayy district, located 6 km south of the brick kilns. Site 5 is situated in Al-Battar, close to the Kut-Baghdad road, and is under the influence of heavy traffic exhaust and vehicular exhaust (Figures 1).



Figure 1. Map of sampling sites in Wasit Province, Iraq.

2.2 Sample collection

In this study the *Lohmann Brown* breed of laying hens was employed as it was the most popular breed used on production farms in the study area. In February 2026, 30 laying hens were sampled with 6 hens per of the 5 designated sites. The age of the birds selected (72-80 weeks) was used to assure uniformity of physiological and productive status among all study sites. Feathers were collected from each laying hen, from breast and wings, and carefully placed in polyethylene containers.

2.3 Analysis of samples

Washing Procedure: The collected feather samples were randomly divided into two major groups. The first group was not washed, to quantify external atmospheric deposition. The second group consisted of an intensive washing sequence aimed at removing external pollutants without leaching internal components: feathers were rinsed 3 times with deionized water, then washed alternatively with pure acetone and deionized water (1:1 v/v) (Grúz *et al.*, 2019), and finally rinsed with deionized water. The washed feathers were oven-dried at 60-80°C for 24 h to constant weight (Yamac *et al.*, 2016).

Digestion Procedure: Breast and wing feathers from each bird were dried, cut into pieces < 2 mm with stainless steel scissors, and pooled into a composite sample to ensure adequate biomass. An aliquot of 0.5 g of each pooled sample was accurately taken in a clean 50 mL borosilicate glass beaker. A concentrated acid mixture (10 mL 65% HNO₃ and 2 mL 30% H₂O₂) was used for wet digestion (Muralidharan *et al.*, 2004). The beakers were covered with watch glasses and put on a digitally controlled hot plate at 90-100°C. Heating was continued until the organic matrix had been completely destroyed, as indicated by the disappearance of dense brown/black nitrous oxide smoke and the formation of a clear, colorless solution with faint white fumes. The digests were cooled to room temperature, filtered through Whatman No. 42 filter paper, quantitatively transferred to volumetric flasks, and diluted to a final volume of 25 mL with deionized water. The samples were stored at -20°C until ICP-OES analysis.

2.4 Heavy metal determination

Samples were analysed for the quantification of Pb, Ni and Cd by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Spectro Arcos, SPECTRO, Germany). The ICP-OES instrumentation and operating conditions are presented in Table 1. The standard solutions of the metals were prepared from the standard stock solution at three different concentrations 100, 500 and 1000 µg. kg⁻¹. Dilution was made with distilled water. The calibration curve was constructed by injection of standards and blank solutions in the ICP-OES. The certified reference material, ERM-CA713, was used for quality control of the trace elements analysis. Also prepared spiked samples. A recovery study was conducted by spiking the tested samples with different concentrations of a multi-element standard solution (Mix standard CRM: 92,091 Supelco LOT BCCB9855, TraceCERT®, elements, 10 mg/L in nitric acid, element standard CRM: 28,941 Supelco, LOT BCCB8927, 1000 mg/L element in nitric acid) and re-measuring the samples. The rates for heavy metal removal were between 95.2 % for Ni and 98.5 % for Cd. Table 2 shows the correlation coefficients (R²), wavelength, limit of detection (LOD), limit of quantification (LOQ) and recovery rates of the studied heavy metals. The samples were analyzed in the laboratories of the Department of Environment and Water / Ministry of Higher Education and Scientific Research.

Table 1. Instrumental and operational conditions of ICP-OES

Parameters	Value/type
RF power	1400 W
Plasma gas flow rate	14.5 L/min
Auxiliary gas flow rate	0.9 L/min
Nebuliser gas flow rate	0.85 L/min
Sample uptake time	240s
Rinse time	45 s
Initial stabilization time	45 s
Measurement Replicates	3
Pump rate	15 rpm
Frequency of RF generator	27.12 MHz
Type of spray chamber Cyclonic	Modified CCD Lichte

Table 2. ICP-OES analysis results (n=3): correlation coefficients (R^2), wavelength, limit of detection (LOD), limit of quantification (LOQ) and recovery rates

Metals	R^2	Wavelength (nm)	LOD (ug.kg ⁻¹)	LOQ (ug.kg ⁻¹)	Recovery rates	RSD %
Pb	0.9995	220.353	1	3	96.8 %	2.50
Cd	0.9997	214.439	0.3	0.9	98.5 %	2.01
Ni	0.9996	231.604	1.5	4.5	95.2 %	3.00

2.5 Statistical Analyses

Data were analyzed using SPSS statistical software (version 23) shown as mean $\pm$ standard deviation. And used one-way analysis of variance (ANOVA) and Duncan's multiple range test (p-value < 0.05) for between study sites comparisons. The Pearson correlation coefficient was used for comparison of the elements in washed and unwashed feathers.

3. Results and Discussion

3.1 Heavy metal concentrations in feathers before and after the washing process

Heavy metals are dangerous pollutants because of their ability to accumulate in living organisms' tissues and biomagnification in the food chain, causing negative effects on the ecosystem and warning of a potential risk to living organisms' health (Almalki et al., 2019). Three heavy metals (lead, cadmium and nickel) were determined as part of the study. The metals were selected because of their high toxicity and severity. The order of metals based on their mean concentration exhibited at the study areas for washed feathers was (Figures 2, 3) lead > nickel > cadmium, the same as for unwashed feathers; thus, washing did not significantly affect the concentration profile of these metals.

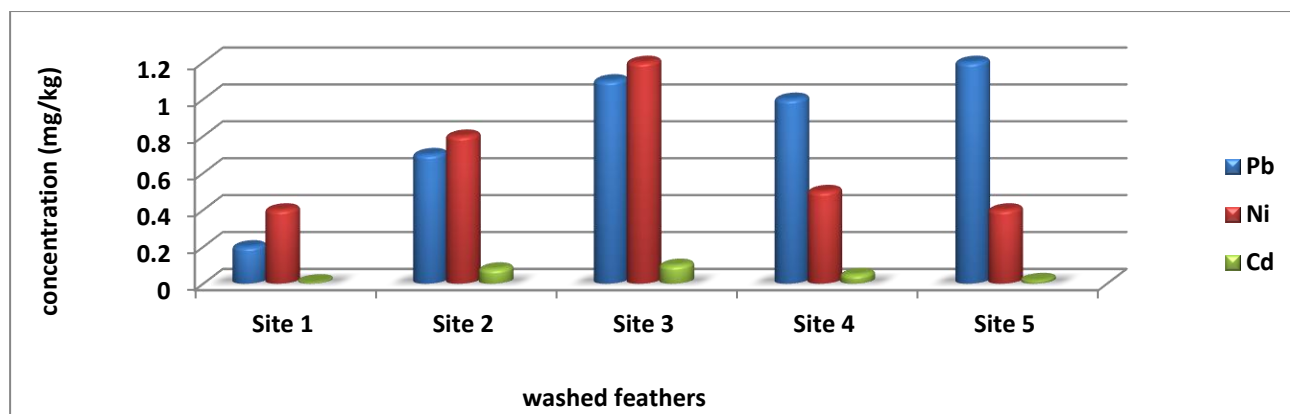


Figure 2. The concentration of metals in the washed feathers (in mg/kg dry weight) in the study areas.

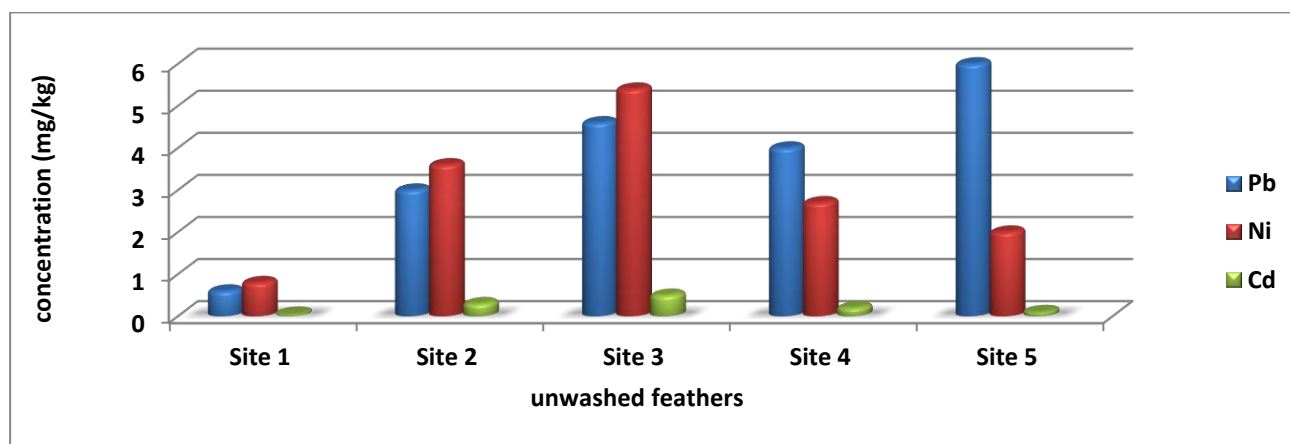


Figure 3. The concentration of metals in the unwashed feathers (in mg/kg dry weight) in the study areas.

3.2 Lead

Pb occurs naturally in the environment in many places and is not essential to ecosystem function, and is toxic from direct or indirect exposure (Johansen et al., 2004). The concentrations of feathers are often correlated to the concentration found in internal tissues, and thus represent the total body burden (Burger, 1993). Washed feathers contained an average concentration of lead of between 0.2 and 1.2 mg kg⁻¹ dry weight from different site areas (Table 3). The average lead concentrations for sites 5 and 1 were highest and lowest, respectively. The results obtained by using the ANOVA test, showed that there is significant difference between the sites being investigated. There was a strong significant correlation between washed and unwashed feathers ($r = 0.812$; $p < 0.01$) (Table 5). When the feathers were not washed, the highest average lead content was 6 mg/kg dry weight at site 5 and the lowest content of lead was 0.6 mg/kg dry weight at site 1 (Table 2). The results showed that there were differences between the sites studied.

The element lead has a higher concentration than nickel and cadmium due to its multiple sources and its high usage in industrial activities. A few studies showed that the lead concentration in the dust and air in Al-Kut was 3.19 $\mu\text{g}/\text{m}^3$, which is almost six times the lead concentration limit set by

the European Agency for the Management and Control of Air Research and Information (EMEA) (Al-Shammari, 2020; EEA, 2017). This major rise in sites 5 and 3 is primarily attributable to vehicular emissions which are a result of the high number of vehicles in the region, high use of leaded fuel and Zubaidiyah power plant. Lead containing particulate emissions from fossil fuel burning facilities and emissions from industry can be released to the atmosphere and deposited in the surrounding environment (Markert, 2007). The concentrations in feathers are mainly due to the continued use of leaded gas (Movalli, 2000).

Table 3. The mean and standard deviation (SD) of heavy elements for Laying Hens within the study area for washed feathers

Site	Descriptive statistics	Lead mg/kg	Nickel mg/kg	Cadmium mg/kg
Site 1	Mean	0.20	0.40	0.01
	SD	0.01	0.02	0.001
		d	c	d
Site 2	Mean	0.70	0.80	0.08
	SD	0.05	0.03	0.002
		c	b	a
Site 3	Mean	1.10	1.20	0.10
	SD	0.08	0.06	0.002
		ab	a	a
Site 4	Mean	1.00	0.50	0.05
	SD	0.06	0.01	0.001
		b	c	b
Site 5	Mean	1.20	0.40	0.02
	SD	0.03	0.03	0.001
		a	c	c
	P-value	< 0.001	< 0.001	< 0.001

Note: Means followed by different letters (a, b, c, d) in the same column are significantly different at $P < 0.05$ level, according to Duncan Multiple Range Test.

Table 4. The mean and standard deviation (SD) of heavy elements for Laying Hens within the study area for unwashed feathers

Site	Descriptive statistics	Lead mg/kg	Nickel mg/kg	Cadmium mg/kg
Site 1	Mean	0.60	0.80	0.05
	SD	0.02	0.02	0.001
		e	d	d
Site 2	Mean	3.00	3.60	0.30
	SD	0.20	0.25	0.02
		d	b	b
Site 3	Mean	4.60	5.40	0.50
	SD	0.35	0.40	0.02
		b	a	a
	Mean	4.00	2.70	0.20

Site 4	SD	0.30	0.18	0.01
		c	c	c
Site 5	Mean	6.00	2.00	0.10
	SD	0.45	0.15	0.008
		a	c	c
	P-value	< 0.001	< 0.001	< 0.001

Note: Means with the same letter (a, b, c, d, e) in a column are not significantly different ($P < 0.05$) by Duncan Multiple Range Test.

3.3 Nickel

Nickel is a highly toxic and carcinogenic heavy metal which causes oxidative stress and DNA damage in birds, which results in reduced growth and immune responses (EEA, 2017).

The results indicated that the lowest concentration of nickel in washed feathers was 0.4 mg/kg dry weight in the site 1 and site 5 and the highest concentration of nickel in washed feathers was 1.2 mg/kg dry weight in the site 3 and in washed feathers as shown in Table 1. Based on the results of the statistical analysis, the study sites were significantly different. As for unwashed feathers, average concentrations of nickel were ranged between 0.8 - 5.4 mg/kg dry weight in the study sites, respectively (Table 4). The results of the statistical analysis showed that there were significant differences between the study sites. A strong significant correlation was obtained between nickel in washed and unwashed feathers ($r = 0.845$; $p < 0.01$) and moderate significant correlation between nickel in unwashed and lead in washed feathers ($r = 0.315$; $p < 0.01$) and moderate correlation between nickel and cadmium ($r = 0.945$; $p < 0.01$) in unwashed feathers (Table 5).

3.4 Cadmium

Cadmium is one of the most dangerous elements as it is highly toxic and remains persistent in the environment for a long time, affecting various biological processes such as the endocrine system, renal function, reproduction, molting, hemoglobin synthesis and growth (Battaglia *et al.*, 2003; Hui, 2002).

In the study sites, the Average concentrations of cadmium in their washed feathers were between 0.01-0.1 mg/kg dry weight (Table 3). Results indicated that there were significant differences between the study sites. The mean Cd concentration of unwashed feathers was the highest at site 3 with 0.5 mg/kg dry weight of feathers; the lowest was at the site 1 with 0.05 mg/kg (Table 4). The results of the statistical analysis showed that there were significant differences between the study sites. There was a significant and positive correlation between Ni and Cd and moderate correlation between Ni and Pb in unwashed feathers ($r = 0.945$ and 0.612 , respectively, $p < 0.01$).

Table 5. Correlation coefficient of washed/unwashed feathers for heavy elements

	Pb washed	Pb unwashed	Ni washed	Ni unwashed	Cd washed	Cd unwashed
Pb washed	1	0.812**	0.231	0.315*	0.774**	0.520*
Pb unwashed		1	0.442	0.440	0.615*	0.612*
Ni washed			1	0.845**	0.792**	0.618*
Ni unwashed				1	0.852**	0.945**
Cd washed					1	0.814**

Note: **= Significant correlation at $P < 0.01$. *= Significant correlation at $P < 0.05$.

The results showed that heavy metal concentrations (Pb, Ni, and Cd) varied significantly in spatial variations between the five study sites. The highest level of contamination was consistently found in the industrial areas, around the Al-Zubaidiyah Thermal Power Plant (Site 3), Al-Ahdab Oil Field (Site 2), Al-Hayy Brick Kilns (Site 4) and the Kut-Baghdad Highway (Site 5). In contrast, the Al-Kardhiya agricultural area (Site 1) showed low burdens of elements. This geographical phenomenon clearly reveals the direct effects of the heavy industrial activities in Wasit Province, which are characterized by the production of substantial amounts of particulates into the air that contain metals, which subsequently settle on the surrounding environment (Markert, 2007).

There was a highly significant difference ($p < 0.001$) between unwashed and washed feathers for all the elements analyzed statistically. The sharp decrease in concentrations after the washing process (which in many samples is more than 70 – 80%) clearly indicates statistical evidence that most of the contamination is exogenous and caused by atmospheric deposition. Feathers have a complex physical structure that acts as a biological "trap" for airborne pollutants dispersed from industrial stacks and oil flares (Borghesi, 2017).

In addition, the high correlation coefficients (r) obtained in the industrial sites for the washed and unwashed condition (especially for lead and nickel) suggests that there is chronic environmental exposure. This indicates that long-term exposure to air pollution produces deposition on the surface as well as endogenous deposition through the inhalation of polluted dust or ingestion of dust contaminated by fallout, from feed (Lodenius and Solonen, 2013). These results thus confirm the susceptibility of poultry in these areas as bioindicators, and that the main medium of transfer of pollutants from industrial sources to the local food chain is air.

Note that growth rates and surface areas exposed to atmospheric deposition are different physiological characteristics of breast and wing feathers. In this study, these feather types were pooled to represent the cumulative bioaccumulation burden per hen and to satisfy the mass requirements for ICP-OES analysis. The composite sampling approach was effective at capturing the overall spatial patterns of contamination across sites, but future studies aiming to distinguish between recent atmospheric deposition and historical dietary intake should consider the separate analysis of flight and body feathers.

4. Conclusion

The results of the research showed the possibility of using bird feathers in the biomonitoring of environmental pollution. It is preferable to use unwashed feathers of birds for environmental monitoring of heavy polluting elements in the atmosphere, while washed feathers are used to identifying contaminated elements for food and water. Biomonitoring can be carried out with the bird studied using unwashed feathers for external contamination with heavy elements. The amount of these elements (lead, nickel and cadmium) found in the feathers of birds and their high concentration in the study area suggests that laying-hen feathers showed preliminary potential as environmental bioindicators.

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