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DETECTION AND COMPARATIVE ANALYSIS OF HEAVY METAL TOXICITY IN BIRDS

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Abstract *Heavy metals are defined as metals with a density of more than 5 to 6 g/cm³. The present study determined the effect and concentration of heavy metals in the organs of pigeons and crows collected from Lahore and Pakpattan. Metal toxicity was determined in pigeons' and crows' vital organs (kidney, lungs and liver). Atomic Absorption Spectrophotometer determined heavy metals concentration. The data was observed by applying ANOVA and mean±SD. Heavy metals analysis showed significantly high concentrations of Cadmium (27.33±0.57) and lead (16.83±0.14) mean values, respectively. There was a low concentration of cadmium (22.83±0.14) and lead (4.83±0.14) mean values, respectively. A probability value at ($p<0.05$) showed significant differences among the different organs of pigeons and crows. The samples showed high bioaccumulation of heavy metals in the organs compared to Pakpattan.*

Keywords: *Heavy metals; Bioaccumulation; Spectrophotometer; Organs; Cadmium; Lead*

Introduction

Metals with a density greater than 5 to 6 g/cm³ are classified as heavy metals. When certain heavy metals are discovered in large concentrations, they harm plants and animals. The concentrations of heavy metals in this environment are estimated in parts per million or parts per billion, as well as dry and wet weights (Hembrom et al., 2020). Heavy metals like lead, mercury, cadmium, and arsenic, among others, are among the most hazardous to humans. Because heavy metals accumulate in the body and create bioaccumulation, they are dangerous. Heavy metal toxicity in food and water is a major environmental issue that can harm the exposed species (Sonone et al., 2020). Heavy and trace metals such as Zn, Cu, and Fe can be found in our surroundings. Metals such as lead, arsenic, and cadmium are toxic to organisms that come into touch with them (Rahman and Singh, 2019). After processing significant volumes of harmful chemicals regularly, many industries impact living organisms in the surrounding environment (Ali et al., 2019). As a result of the repercussions, animals, particularly pedigree animals, may perish (Jansson and Laikre, 2018). Like other developing countries, Pakistan faces major environmental problems, including trace metal contamination in various sections of the country (Khalid et al., 2018). Trace metals (Zn, Pb, Co, Mn, Cd, Cu, and others) are common environmental pollutants that remain in the environment, causing food chain accumulation and the potential for major health consequences in humans and wildlife. Industrial waste dumping, agriculture runoff, mining and mineral processing, volcanic outburst dust exposure, and air movement have all been linked to environmental pollution in various compartments (Kour et al., 2021). Researchers determined that the combined effects of cadmium and arsenic resulted in deadly consequences by studying the blood biochemistry of mice. Metal contamination has a long-term effect, and excessive ingestion can harm a bird's health (Bauerová et al., 2020).

Aims and Objectives

1.To estimate the concentration of heavy metals in pigeons' and crows' organs, kidneys, liver, and lungs.
2.To compare heavy metals' analysis in Lahore and Pakpattan birds.

Experimental Methods

The research was conducted at Lahore Garrison University Lahore. Experimental work was performed at PCSIR (Pakistan Council of Scientific and Industrial Research) Lahore. The study was conducted to estimate the level of heavy metal concentration in some vital organs of pigeons and crows, which were collected from Lahore and Pakpattan.

Sample Collection and Preparation

The pigeons and crows were collected from Lahore and Pakpattan. The birds were slaughtered, and fresh samples of the liver, kidney and lungs were separated for experimental work. The mentioned organs were cleaned and washed with distilled water. Then were cut into small, sizeable pieces with a knife. Then, each sample was kept in a separate polythene bag and labeled. Samples were placed in thermoses filled with

ice cubes and brought to the CEPS(Center for Environmental Protection Studies) lab of PCSIR Lahore. Samples were stored in the freezer of CEPS lab at –10 C° for further analysis.

Heavy metals analysis

In the study, two heavy metals were analyzed. Determination of cadmium concentration in pigeons' and crows' organs' samples. Determination of concentration of lead in pigeons and crow organs samples.

Steps involved in the assay of metals

- Taking a representative sample from the large amount of data collected during testing.
- Acid digestion of Samples
- Determination of Cadmium and Lead by Atomic Absorption Spectrophotometer.

Acid digestion of Samples

In the study, commonly used methods of digestion of samples can be broadly grouped into wet digestion, dry ashing, and then digestion, such as microwave digestion.

Wet Digestion

The most used method to digest a wide variety of food samples was wet digestion. The general procedure given below was adopted for the digestion of samples. All the digestion procedures were performed in the fuming hood, which had a proper exhaust system.

Procedure

Took 2g of the sample into a macro-kjeldhal digestion flask. Added 20ml concentrations of nitric acid and heated the flask contents to reduce the volume. The solution was cooled, 10ml of concentrated sulphuric acid was added, and it was boiled again. More quantity of nitric acid was added whenever the contents began to blacken. When the sample became Clear and white fumes evolved, and then heating was stopped. At this stage, the solution was cooled, and then add 10ml saturated ammonium oxalate solution was heated until copious white vapors were created. The oxalate treatment aids in the removal of the yellow color caused by nitro compounds, lipids, and other substances, resulting in a colorless final solution. Before starting with the metals test, all traces of nitric acid must be eliminated. At the same time, a blank was created. Nitric acid, sulphuric acid, perchloric acid, and hydrogen peroxide were the most regularly utilized oxidants. Combinations containing two or more of the following chemicals is advised due to their inherent advantages (Figure 1 & 2).

Determination of cadmium and lead in pigeons and crows' organs by Atomic Absorption Spectrophotometer Principle

The method uses an atomic absorption spectrophotometer to determine an analyte's concentration in a sample. By absorbing a certain amount of energy, the atom's electrons in the atomizer can be promoted to higher orbitals (the excited state) for a brief amount of time (nanoseconds). This energy level, or wavelength, was unique to an electron transfer in a certain element. Figure 1 show the apparatus of this experiment. Determination of cadmium and lead in pigeons and crows organs by Atomic Absorption Spectrophotometer followed by (Chen and Teo, 2001; Sisay et al., 2019).

Figure 1 Showing Hot plate apparatus for digestion of samples

Element Wavelength Flame-Gases

Cadmium 279.5 Air-acetylene and Lead 232.0 Air-acetylene (Figure 2).

Figure 2 Showing Atomic Absorption Spectrophotometer for heavy metals

Result and Discussion

The present study was conducted at CEPS, PCSIR in the Department of Biology at Lahore Garrison University. During this study, Cadmium and Lead concentration was determined in birds' organs (Crows and Pigeons) collected from Lahore and Pakpattan.

Cadmium and Lead concentration in Pigeons organs

The data presented in Table 1 showed the mean value of cadmium and lead concentration in different organs viz kidney, lungs, and liver in pigeons. The samples of pigeon's organs collected from Lahore and Pakpattan. Pigeon organs showed significantly variable responses to cadmium and lead concentrations.

Columbidae (Pigeon)

The cadmium and lead concentration was determined in different organs of Columbidae (Pigeon), and data is presented in Tables 1 & 2. Maximum concentration of cadmium and lead were observed in lungs followed by the liver and kidney, with average values of ug/g, respectively. This result indicated a significant difference between lungs and liver. The results from pigeons *Columbidae* were computed as significant, indicating that *Columbidae* species showed different concentrations of cadmium and lead. The overall pattern of concentration of cadmium in organs of experimental Columbidae species was observed as high concentration of Cd was in the liver, as shown in table 5&6. The lowest concentration of Cd was in the kidney as liver (25.83±4µg/g), lungs (25.83±0.14µg/g) and kidney (25.67±0.57µg/g) with the mean value in Pakpattan and lead in organs of experimental Columbidae species was observed as high concentration of Pb in lungs and lowest concentration of Pb in kidneys such as lungs (12.83±0.14µg/g), liver (8.33±0.57µg/g) and kidney (7.5±0.43µg) with the mean value in Pakpattan and in Lahore the highest concentration of Cd was in the lungs and lowest concentration of Cd was in the liver such as lungs (27.33±0.57µg/g), kidney (26.83±0.14µg/g) and liver(25.83±0.14µg/g) with the mean values in Lahore and in Lahore the highest concentration of Pb was in the lungs and lowest concentration of Pb was in the liver such as lungs (12.66±0.28µg/g), kidney(7.66±0.28µg/g) and liver (7.58±0.38µ g/g) with the mean values in Lahore respectively (figure 3 & 4).

Cadmium and Lead concentration in crow's organs

The data in tables 3 & 4 showed mean values of cadmium and lead in different organs viz kidney, lungs, and liver in crow. The samples of crows' organs collected from Lahore and Pakpattan. Crows' organs showed significantly variable response to cadmium and lead concentration.

Corvus splendens (Crow)

The cadmium and lead concentrations were determined in different organs of *Corvus splendens*, and the data is presented in Table 7 & 8. Maximum concentration of Cadmium and Lead was observed in lungs followed by liver and kidney with average values of ug/g respectively. This result indicated significant difference between lungs and liver. The results from Corvus splendens were computed as significant, indicating that *Corvus splendens* species showed different concentrations of cadmium and lead. The overall pattern of concentration of cadmium and lead in organs of experimental Corvus splendens species was observed as the highest concentration of Cd was in the liver and lowest concentration of Cd was in the lungs such as liver (25.66±0.28µg/g), kidney (25.66±0.28µg/g) and lungs with the mean values in Pakpattan and the highest concentration of Pb was in the liver and lowest concentration of Pb was in the kidney such as liver (7.33±0.57µg/g), lungs (7.33±0.57µg/g) and kidney (6.33± 0.57µg/g) with the mean values in Pakpattan and Lahore the highest concentration of Cd was in the lungs and the lowest concentration of Cd was in the liver such as lungs (27.33±0.57µg/g), kidney (26.33±0.57µg/g) and liver (26.33±0.57µg/g) with the mean value and the highest concentration of Pb was in the liver and the lowest concentration of Pb was in the kidney such as liver (16.83±0.14µg/g), lungs (8.33±0.57µg/g) and kidney (7.66±0.28µg/g) with the mean values in Lahore respectively (figure 5 & 6).

Statistical analysis

Mean value of individual metals was compared with one-way ANOVA. The variation was observed within the group. The significant values are represented by asterisk(*).

Pigeons

Descriptives table 1

ANOVA table 2

Crow

Descriptives table 1

ANOVA table 2

Table 1: Showing the Mean±SD of the concentration of heavy metals Cd and Pb in pigeons organs (Kidney, Lung, Liver) collected from Pakpattan.

Samples	Kidney		Lung		Liver	
	Cd	Pb	Cd	Pb	Cd	Pb
A	25.67±0.57	7.33±0.28	25.83±0.14	6.33±0.57	25.83±0.14	7.33±0.57
B	25.33±0.28	6.33±0.57	25.83±0.14	6.66±0.28	25.83±0.14	6.83±0.14
C	25.83±0.14	7.33±0.57	25.83±0.14	8.66±0.28	25.83±0.14	7.66±0.28
D	25.67±0.57	7.5±0.43	25.83±0.14	12.83±0.14	25.83±0.14	8.33±0.57
E	25.83±0.14	6.66±0.28	25.83±0.14	7.66±0.28	25.83±0.14	6.58±0.38

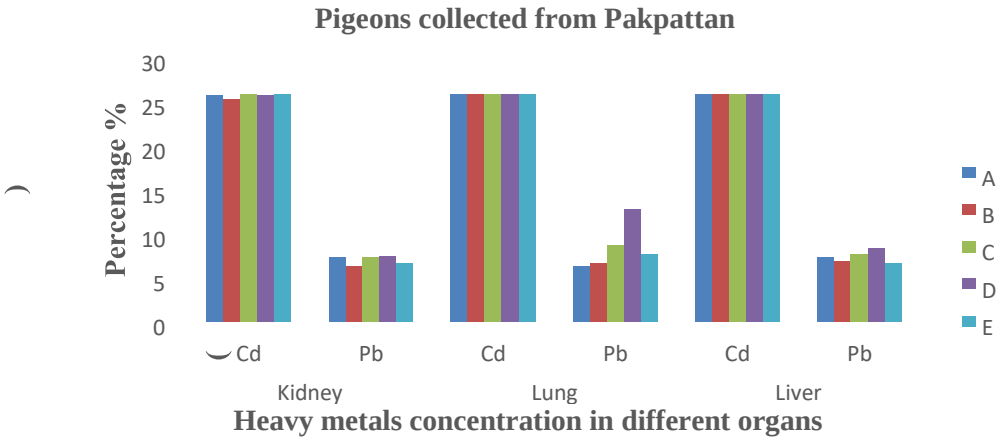

Figure 3: Showing the comparison of heavy metals among different organs

Table 2: Showing the Mean±SD of the concentration of heavy metals Cd and Pb in the pigeons organs (Kidney, Lung, Liver) collected from Lahore

Samples	Kidney		Lung		Liver	
	Cd	Pb	Cd	Pb	Cd	Pb
A	26.33±0.57	7.58±0.38	25.33±0.57	7.33±0.57	25.66±0.28	7.66±0.28
B	25.66±0.28	5.66±0.28	25.83±0.14	6.83±0.14	25.66±0.28	7.33±0.57
C	25.83±0.14	6.33±0.57	26.33±0.57	7.66±0.28	25.83±0.14	6.66±0.28
D	26.83±0.14	6.5±0.43	25.66±0.28	8.33±0.57	25.83±0.14	6.33±0.14
E	26.33±0.14	6.83±0.14	27.33±0.57	12.66±0.28	25.83±0.14	6.66±0.28

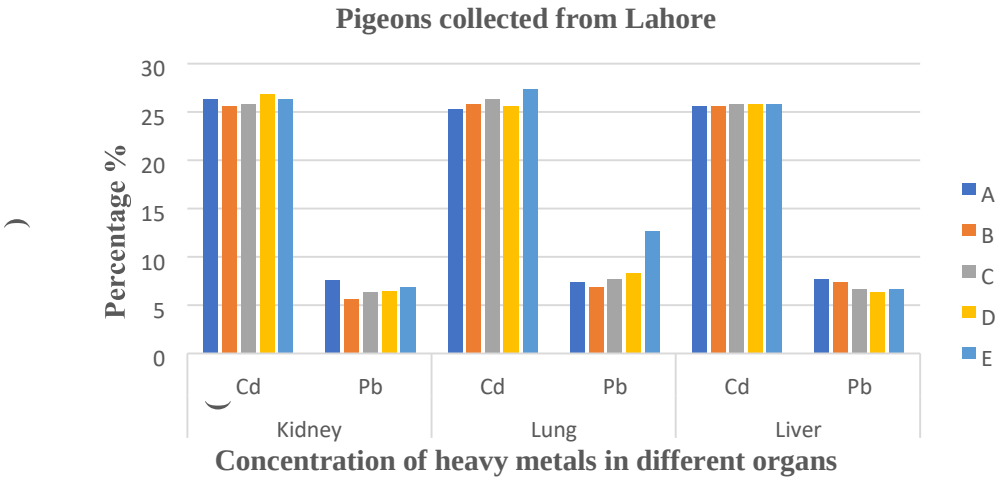

Figure 4: Showing the comparison of heavy metals among different organs

Table 3: Showing the Mean±SD of the concentration of heavy metals Cd and Pb in the crow organs (Kidney, Lung, Liver) collected from Pakpattan

Samples	Kidney		Lungs		Liver	
	Cd	Pb	Cd	Pb	Cd	Pb
A	23.33±0.57	5.33±0.57	24.66±0.28	6.33±0.57	24.66±0.28	5.66±0.28
B	25.66±0.28	6.5±0.43	22.83±0.14	5.66±0.28	24.83±0.14	6.33±0.57
C	24.66±0.28	5.83±0.14	23.33±0.57	7.33±0.57	25.33±0.57	7.33±0.57
D	22.83±0.14	6.33±0.57	24.33±0.57	6.66±0.28	23.83±0.14	5.83±0.14
E	23.83±0.14	5.66±0.28	23.66±0.28	5.83±0.14	25.66±0.28	4.83±0.14

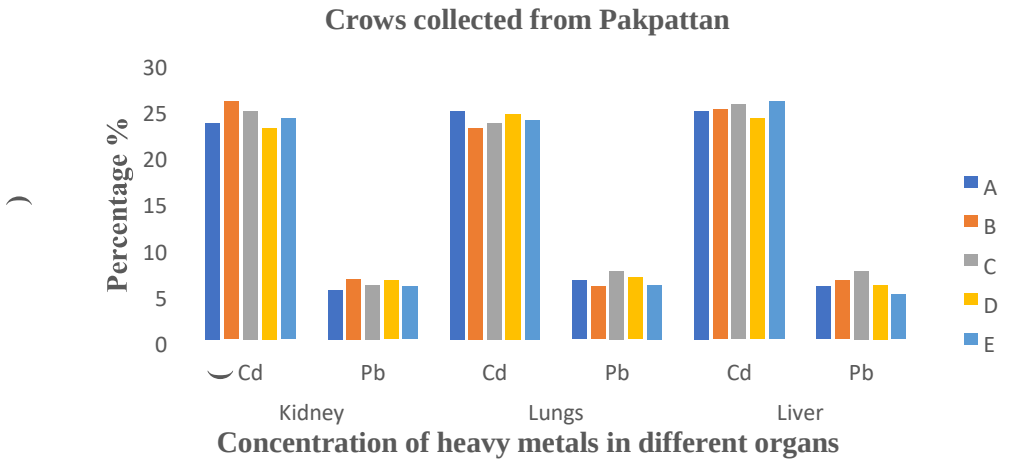

Figure 5: Showing the comparison of heavy metals among different organs

Table 4 : Showing the Mean±SD of the concentration of heavy metals Cd and Pb in the crow organs (Kidney, Lung, Liver)collected from Lahore.

Samples	Kidney		Lung		Liver	
	Cd	Pb	Cd	Pb	Cd	Pb
A	25.5±0.43	6.66±0.28	27.33±0.57	8.33±0.57	24.33±0.57	16.83±0.14
B	25.83±0.14	7.66±0.28	25.66±0.28	7.66±0.28	25.83±0.14	11.66±0.28
C	25.83±0.14	7.33±0.57	26.33±0.57	7.33±0.14	26.33±0.57	9.33±0.57
D	26.33±0.57	6.66±0.28	25.83±0.14	7.33±0.14	25.66±0.28	8.83±0.14
E	25.83±0.14	6.83±0.14	26.66±0.28	5.66±0.28	24.83±0.14	8.33±0.14

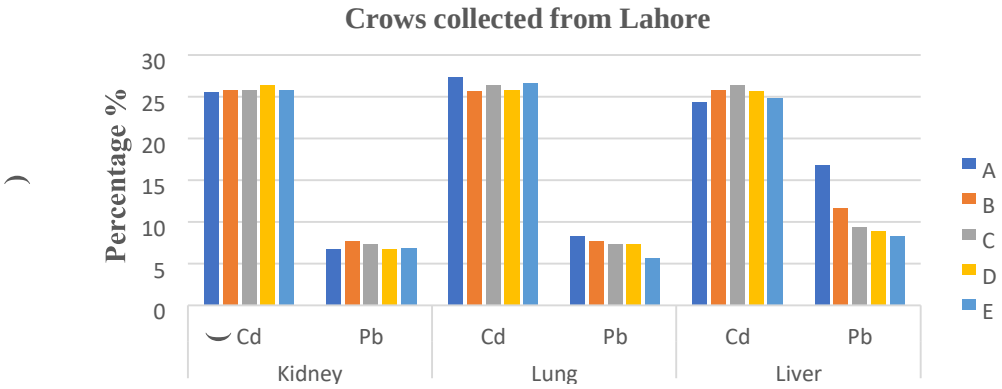

Figure 6: Concentration of heavy metals in different organs

Table 5: Descriptives Pigeons

		N	Mean	Std. Deviation	Minimum	Maximum
kidney	PakpattanCd	5	25.6660	.20416	25.33	25.83
	PakpattanPb	5	7.0300	.50690	6.33	7.50
	LahoreCd	5	26.1960	.46344	25.66	26.83
	LahorePb	5	6.5800	.70317	5.66	7.58
	Total	20	16.3680	9.82548	5.66	26.83
Lung	PakpattanCd	5	25.8300	.00000	25.83	25.83
	PakpattanPb	5	8.4280	2.62434	6.33	12.83
	LahoreCd	5	26.0960	.77864	25.33	27.33
	LahorePb	5	8.5620	2.35471	6.83	12.66
	Total	20	17.2290	9.11341	6.33	27.33
Liver	PakpattanCd	5	25.8300	.00000	25.83	25.83

	PakpattanPb	5	7.3460	.69284	6.58	8.33
	LahoreCd	5	25.7620	.09311	25.66	25.83
	LahorePb	5	6.9280	.54742	6.33	7.66
	Total	20	16.4665	9.58176	6.33	25.83

Table 6: ANOVA (pigeon)

		Sum of Squares	df	Mean Square	F	Sig(p).
kidney	Between Groups	1830.228	3	610.076	2421.273	.*000
	Within Groups	4.031	16	.252		
	Total	1834.259	19			
Lung	Between Groups	1525.877	3	508.626	156.043	.*000
	Within Groups	52.152	16	3.260		
	Total	1578.029	19			
Liver	Between Groups	1741.240	3	580.413	2944.877	.*000
	Within Groups	3.153	16	.197		
	Total	1744.393	19			

Table 7: Descriptives Crow

		N	Mean	Std. Deviation	Minimum	Maximum
kidney	PakpattanCd	5	24.0620	1.12012	22.83	25.66
	PakpattanPb	5	5.9300	.48161	5.33	6.50
	LahoreCd	5	26.0300	.27386	25.83	26.33
	LahorePb	5	7.0280	.44740	6.66	7.66
	Total	20	15.7625	9.57906	5.33	26.33
lungs	PakpattanCd	5	23.7620	.74072	22.83	24.66
	PakpattanPb	5	6.3620	.67132	5.66	7.33
	LahoreCd	5	26.3620	.67132	25.66	27.33
	LahorePb	5	7.2620	.98421	5.66	8.33
	Total	20	15.9370	9.44210	5.66	27.33
liver	PakpattanCd	5	24.8620	.70048	23.83	25.66
	PakpattanPb	5	5.9960	.92075	4.83	7.33
	LahoreCd	5	25.3960	.80423	24.33	26.33
	LahorePb	5	10.9960	3.50183	8.33	16.83
	Total	20	16.8125	8.89556	4.83	26.33

Table 8: ANOVA (crow)

		Sum of Squares	df	Mean Square	F	Sig.(p)
kidney	Between Groups	1736.364	3	578.788	1314.091	.*000
	Within Groups	7.047	16	.440		
	Total	1743.411	19			
lungs	Between Groups	1684.237	3	561.412	928.461	.*000
	Within Groups	9.675	16	.605		
	Total	1693.912	19			
Liver	Between Groups	1446.496	3	482.165	135.364	.*000
	Within Groups	56.992	16	3.562		
	Total	1503.488	19			

Discussion

The goal of the present study was to determine the level of heavy metals (Cadmium and Lead) in the more polluted area Lahore and less polluted area Pakpattan in the organs (liver, kidney and lungs) of avian species. For this purpose, common avian

species i.e Pigeon (*Columbidae*) and crow (*Corvus splendens*), have been captured from the sampling sites Lahore and Pakpattan. The birds were captured by using mist nets. As a result of urbanization and industrial activities, large amounts of contaminants have been regularly injected into the ecosystem. Heavy metals affect the birds, as well as other organisms. Metals, for example, have been discovered to decrease bird immune systems, raise territorial aggression, cause reproductive dysfunction, increase disease susceptibility, and stress alterations in behavioral patterns. Ingestion of sediments containing lead and cadmium is a common cause of nonparticulate lead and cadmium exposure due to urbanization and industrial processes. Not only can ingestion of lead-contaminated sediments, particularly near automobiles and businesses, cause particle lead and cadmium exposure(Natasha et al., 2020). Moreover, birds' lead and cadmium contact has arisen in urban environments from airborne sources(Levin et al., 2021). Bioaccumulation is greater in the highly polluted area Lahore when air is heavily contaminated with metals. Heavy metal exposure is increased due to industrial waste material and vehicles in the urbanized areas. Bioaccumulation is comparatively less in the less polluted area Pakpattan because the air of Pakpattan is less contaminated with metals. Carnivorous birds were found to have higher amount of metals as compared to omnivorous and insectivorous birds. The above study concludes that in Punjab province, it can be due to agriculture and industrialization high risks of heavy metal contamination (Rashid et al., 2018). To examine metal levels and the possible damage to birds and people who consume them, researchers measured zinc (Zn), lead (Pb), nickel (Ni), and cadmium (Cd) amounts in the liver, kidney, and lungs of birds of Punjab province. Lahore and Pakpattan showed significant results against heavy metals concentration in some vital organs(Imtiaz et al., 2020).

In the present study, heavy metal cadmium and lead were detected in the birds (Pigeon& Crow) organs (kidney, liver, lungs), collected from two different sites Lahore and Pakpattan. During this study in birds organs samples, the highest concentration of cadmium and lead was detected in the crows' organs collected from Lahore, and the lowest concentration of cadmium and lead was detected in crow organs collected from Pakpattan because Lahore is highly urbanized and industrialized compared to Pakpattan. Almost all species studied showed significant increases in adult and nestling populations throughout the same gradient. Only two previous studies evaluating lead contamination in birds in urban areas are known to us(Levin et al., 2021), and both produced similar results.

The annual survival of adult birds, and it's feasible that birds could succumb to the deadly effects of lead poisoning over longer periods (Hanley et al., 2022) showed significant comparison to our study. Contamination with lead and cadmium in urban surroundings was just one of the numerous challenges that birds living in this land-use type face. Other environmental factors include toxins like mercury, as well as the replacement of native plants with exotic vegetation(Chapman et al., 2019), noise pollution (and pedestrian disturbance are all examples of free–roaming cats as predators(Fardell et al., 2021). These elements have been shown to influence bird roosting, breeding, and foraging behavior.

According to the present study, it was observed through the heavy metals analysis by atomic absorption spectrophotometer that the high concentration of heavy metals affects the birds' breeding behavior, and the birds' stress level was also increased due to high accretion of heavy metals in bird's organs. Pb, Cd levels in the liver and kidney differed as a function of dietary environments, according to a study by; median values were significantly greater in invertebrate predators than predators and omnivorous species because the predators or omnivorous species can regulate their internal metal concentration better then invertebrates predator species and also have higher body mass to surface ratio(Liu et al., 2019). Short-distance migrants exhibited significantly higher median heavy metal levels of lead is >6µg/g in their liver and kidney and long-distance migrants exhibited>5µg/g in their liver and kidney.

Conclusion

It was eventually concluded that heavy metals are very dangerous for birds because they cause bioaccumulation in the body of birds. The heavy metals contamination affects the birds. It was concluded that the samples of pigeons and crows collected from Lahore showed high bioaccumulation of heavy metals, which were selected for the experimental study of heavy metals analysis. The highest concentration of cadmium (27.33±0.57>26.83±0.14>25.83±0.14) in the pigeons' organs and (27.33±0.57>26.33±0.57> 26.33±0.57) in crows organs and lead (12.66±0.28> 7.66±0.28>7.58±0.38) in pigeons organs and (16.83±0.148.33±0.57> 7.66±0.28) in crows, organs were detected which were collected from Lahore. The lowest concentration of Cadmium (25.83±0.14>25.83±0.14>25.67±0.57) in pigeons' organs and (25.66± 0.28>25.66±0.28>24.66±0.28) in crows organs and lead (12.83±0.14>8.33±0.57> 7.5±0.43) in pigeons organs and (8.33± 0.57>7.33±0.57>6.33±0.57) in crows organs was a detective who was collected from Pakapattan. The organs of pigeons and crows, which were collected from Lahore, showed the highest concentration of cadmium and lead compared to Pakpattan due to high pollution, urbanization, and industrialization. The organs of pigeons and crows collected from Pakpattan showed the lowest concentration of Cadmium and lead compared to Lahore due to less pollution and industrialization and urbanization.

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Conflict of interest

There is no conflict of interest among the authors.

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These aspects are not applicable in this paper.

Consent for publication

Not applicable

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