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An Ecotoxicological Study of House Crow in Southern Punjab, Pakistan

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Abstract

Twenty House Crow (*Corvus splendens*) were collected from five districts of Punjab Pakistan such as Rajanpur, Dera Ghazi Khan, Muzaffar Garh, Khanewal and Vehari. After analyzing the samples of birds, it was found that the mean concentrations of metals such as copper, cadmium and zinc were higher in liver samples as compared to kidney samples. Non-significant value of zinc was observed in liver samples (P = 0.175) while in kidney it's significant is (P = 0.040). There was no prominent difference was observed in copper concentration in liver (0.244) and kidney samples (0.236). Cadmium concentration found in liver is (0.162) and in kidney samples (0.057). There was no significant difference was seen in mean values of metals in kidney and liver samples in all study areas. The higher industrial rate in urban areas of Pakistan lead to heavy metal toxicity which is a major problem that is associated with severe health issues. The study was designed to find out the level of certain heavy metals including zinc, copper and cadmium in samples of an urban bird species that is House Crow (*Corvus splendens*). The higher values of these metals and their effects found in birds will help the humans.

Keywords: Cadmium, Copper, Kidney, Pollution, Toxicity, Zinc

1. Introduction

Birds act as bioindicators of environmental pollution. Many birds' species are found at the highest position of the food chain and bio accumulates toxicants. They are sensitive and serve as an early warning function. Egg and egg shell in females act as important indicator for excretion of some trace elements in birds[1,2].

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Heavy metals cause toxicities to living organism. Metallic ions bind to the cell membrane and make it difficult for transport process of materials through the cell wall[3,4]. Metals accumulation levels in birds vary according the biological processes of birds such as growth stage, reproduction, shedding and migration, etc.[5]. Heavy metals have close association with birds related to physiology, development and behavior related issues[6–8]. Birds may be exposed to chemicals in different ways such as direct contact or either ingestion of contaminated water and food such as Mercury (Hg), Cadmium (Cd), Cobalt (Co)[9]. Raptors such as Hawk, Eagle or Owl are good bioindicators because they are found at the top of the food chain and accumulate pollutants to major level[10].

Metal has entered body accumulated in different tissues; its accumulation level is higher in liver and kidney. Female birds have hid away heavy metals in eggs and eggshells[11]. Heavy metals, whether biologically essential or not have the capacity to toxic for organisms above a certain threshold concentration[12]. Heavy metals such as mercury, cadmium and lead have caused many pollution incidents in Japan, Sweden and Iraq[13–15]. This is because that they are extraordinarily toxic[16]. Many metals do not show significant biological function which accumulates in environment due to human activities[17].

Long-term effects of environmental pollutants have increased in recent years and biological monitoring has a satisfactory way to express heavy metal in large quantity and absorption in living organism[18-20].

Non-essential element such as cadmium was correlated with increased industrial exposure[21]. Population declines, reproductive failures, behavioral abnormalities in birds can indicate environmental problem such as presence of heavy metals[22]. Metals accumulation level is much higher in animals than in water or air[23]. It is needed to monitor, evaluate and remediate ecological damage due to environmental contamination[24,25]. Metal ions greatly accelerate in large amounts to turn-over and breakdown of heme which is necessary for cell respiration, energy generation and oxidative biotransformation[26]. They are often referred to as trace elements, as they found in low concentrations in organisms[27].

Cadmium is a non-essential element for living organisms. It accumulates in the body, especially in the kidneys[28]. Its source is burning coal, oils and from wear of vehicle tires[29]. Higher level in the liver and kidney show acute exposure. Its effect on birds is in different ways such as retard growth, reduced egg production and changing behavior[30–32].

Copper is abundantly found in rocks, water and air. The main cause for Cu pollution is production of home tools, metal handling, timber industry and ashes[33]. It is very important element for all living organisms but higher levels may cause severe effects in all biological species[34].
Zinc is the second type of heavy metal poisoning in birds but its poisoning is very rare in most species[35]. Higher level of zinc can be lethal to frog and fish embryos that inhibit the synthesis of DNA[36].

The main objective was to compare metal levels between different areas and to provide daily basis data for comparison with future left over level of metals in house crow (*Corvus splendens*) in the same areas.

2. Materials and Methods

2.1 Sample Collection

Twenty samples of House Crow (*Corvus splendens*) for the present study were collected from five districts, Rajanpur (N 29.6, E 73.6), Dera Ghazi Khan (N 30.06, E 70.63), Khanewal (N 30.20, E 71.99), Vehari (N 30.04, E 72.23), Muzaffer Garh (N 30.05, E 71.14) of Southern Punjab, Pakistan. Live crows were collected randomly from each district. These were weighed, measured and dissected.

2.2 Study Site and Approval

Study was conducted in department of pure and applied biology BZU Multan Pakistan. It was approved from the Board of Advanced Studies and Research and ethical comity of BZU Multan Pakistan. The ethical letter number BZU/ORIC/20.

2.3 Sample Preparations

2.3.1 Preparation of Kidney Solution

Kidney samples (200-415 mg) were digested by reflux condensing method in 5 ml aquarigia (con.HNO_3, HCl) and these samples were diluted with deionized water up till 25 ml volume.

2.3.2 Preparation of Liver Solution

Liver samples (350-420 mg) were digested by reflux condensing method in 5 ml aquarigia (con.HNO_3, HCl) and these samples were diluted with deionized water up till 25 ml volume.

2.4 Statistical Analysis

Results were demonstrated in mean and standard deviation. Data was analyzed by using Analysis of Variance (ANOVA). Data was significant at <0.05.

3. Results

A sum of twenty House Crow (*Corvus splendens*) were analyzed for overall mean metal and trace element concentrations in kidney and liver samples from five districts of southern Punjab, Pakistan (Table 1).

Table 1. Overall mean metal and trace element concentrations in kidney and liver samples of House crow (Corvus splendens) from five disctricts of Southern Punjab, Pakistan (Arithmetic mean, standard deviation, range)

Metals(Liver)	No. of Birds	Mean (µg/g wet wt.)	SD	Range (µg/g wet wt.)
Zn	20	31.53	27.68	1.38-132.8
Cu	20	7.04	5.9	0.39-21.35
Cd	20	44.41	31.96	9.43-83.11
Kidney	20			
Zn	20	29.46	56.79	0.22-170.28
Cu	20	5.13	6.02	0.37-28.75
Cd	20	40.86	56.36	2.41-109.86

3.1 Comparative Analysis of Metals in Liver Samples of House Crow (*Corvus splen dens*) among Five Districts of Southern Punjab, Pakistan

3.1.1 Zinc

There was a significant difference (p = 0.04) in zinc concentration among all five districts of which, the house crow samples of Khanewal has the highest concentration of zinc (80.2 µg/g) followed by Rajanpur (22.7 µg/g), Dera Ghazi Khan (20.56 µg/g), Vehari (18.26 µg/g). However, lowest value of zinc was found in the house crow samples of MuzaffarGarh (15.67µg/g), (Table 2, Figure 1).

3.1.2 Copper

There was no significant difference (p = 0.244) of copper concentration among all five districts. House crow of Muzaffar Garh has the highest concentration of Copper (12.78 µg/g) followed by samples of Khanewal (7.39 µg/g), Vehari (7.26 µg/g), Dera Ghazi Khan (4.13 µg/g) and the lowest value of Cu was found in the samples of Rajanpur (3.67µg/g), (Table 2, Figure 2).

3.1.3 Cadmium

There was no significant difference (p = 0.162) in cadmium concentration appeared among all five districts. House crow samples of Dera Ghazi Khan has the highest concentration of Cadmium (77.9 µg/g) followed by Rajanpur (44.9 µg/g), Muzaffar Garh (42.2 µg/g), Khanewal (28.7µg/g) and the lowest concentration of Cd was observed in house crow samples of Vehari (28.4 µg/g), (Table 2, Figure 3).

3.2 Comparative Analysis of Metals in Kidney Samples of House Crow (*Corvus splen dens*) among Five Districts of Southern Punjab, Pakistan

3.2.1 Zinc

There was no significant difference was seen (p = 0.17) in zinc among all five districts. However house crow of Khanewal should highest concentration of Zn (109.9 µg/g) followed by Muzafar Garh (15.8 µg/g), Vehari (13.7 µg/g), Rajanpur (7.9 µg/g) and the lowest concentration of Zn was observed in the samples of Dera Ghazi Khan (0µg/g), (Table 3, Figure 4).

3.2.2 Copper

There was no significant difference was observed (p = 0.236) in the concentration of copper in all five districts of Southern Punjab, where house crow samples of Muzaffar Garh has the highest concentration of Copper (8.64 µg/g) followed by Khanewal (7.69 µg/g), Vehari (3.71 µg/g), Dera Ghazi Khan (3.35 µg/g) and the lowest concentration is found in the samples of Rajanpur (2.27µg/g), (Table 3, Figure 5).

3.2.3 Cadmium

A significant difference was observed (p = 0.057) in cadmium concentration data from all five districts. House crow of Dera Ghazi Khan has highest concentration of Cadmium (90.04 µg/g) followed by Rajanpur (46.1 µg/g), Muzaffar Garh (40 µg/g), Vehari (15.39 µg/g) with the lowest value found in samples of Khanewal (11.87 µg/g), (Table 3, Figure 6).

Table 2. Mean concentration of metals in Liver samples of House Crow (*Corvus splendens*) from urban areas of five districts of Southern Punjab, Pakistan. Given are means+ SD (Geometric means are given below in parentheses). Comparison is made with one-way ANOV A. All values arc in ppm wet weight.

Location	Zn (JJglg)	Cu (IJg/g)	Cd (IJg/g)
RAJ	22.7± 15.8 (12.39)	I 3.67±2.03 (2.59)	44.9±12 (41_05)
DGK	20.56±0 (20.56)	4.13±0 (4.13)	77.9±0 (77.9)
KWL	80.2±21 .2 (73.2)	7.26±1.04 (7.11)	28.67±2.36 (28.46)
VHR	18.26±9.81 (13.89)	7.39±1.72 (7.01)	28.42±7.46(28.46)
M.G	15.67±5.55 (13.13)	12.78±3.8 3(10.9)	28.42±7.46(26.56)
ANOVA F (P)	3.95(0.04)	1.65 (0.244)	2. 18(0.162)
Toxic level	>200	>1	>3

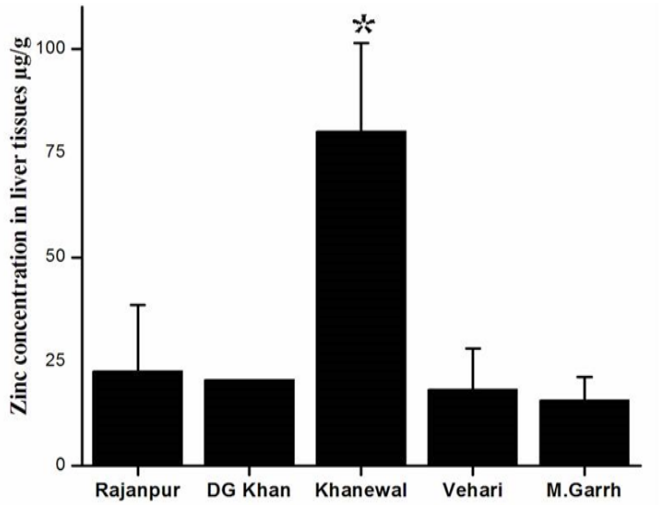


Figure 1. Comparison between mean concentration of Zinc in liver tissues (n=20) of house crow (*Corvus splendens*) from southern Punjab, Pakistan.
Khanewal has the highest mean value of zinc in liver tissues (80.2 µg/g) followed by Rajanpur (22.7µg/g), Dera Ghazi Khan (20.56 µg/g), Vehari (18.3 µg/g) and the lowest value is found in Muzaffar Garb (15.6 µg/g). The range of Zn in the liver is (1.38-132.8 µg/g) and mean concentration of zinc in the liver is (3 1.53 µg/g).

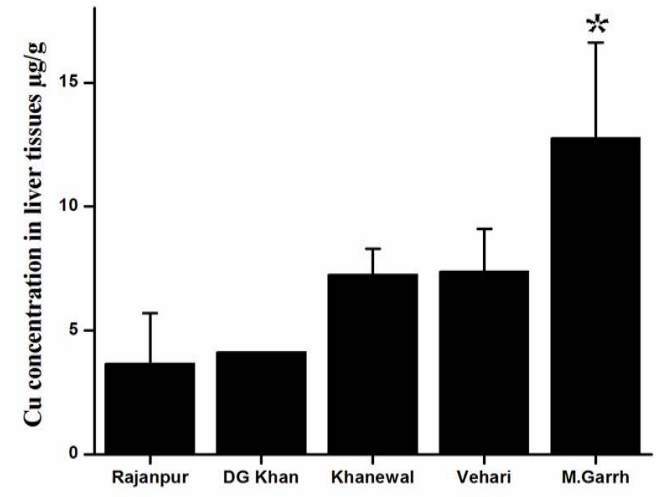


Figure 2. Comparison between mean concentration of copper in liver tissues (n=20) of house crow (*Corvus splendens*) from southern Punjab, Pakistan.
Muzaffar Garb has the highest mean value of copper in liver is (12.78 µg/g) followed by Vehari is (7.39 µg/g), Khanewal (7.26 µg/g), Dera Ghazi Khan (4.13 µg/g) and the lowest value of copper found in Rajanpur (3.67 µg/g). The range of copper in liver is (0.39-2 1.35 µg/g) and means concentration of copper in liver is 7.04 µg/g which clearly indicate the copper concentration above the permissible limits.

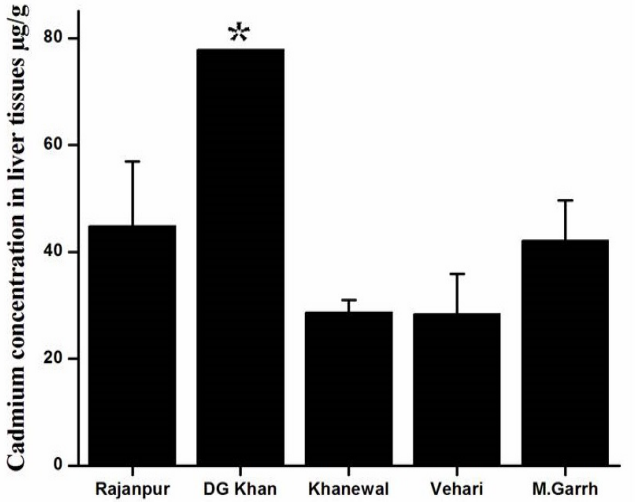


Figure 3. **Comparison between mean concentration of cadmium in liver tissues (n=20) of house crow (*Corvus splendens*) from southern Punjab, Pakistan.**
Dera Ghazi Khan has the highest mean value of cadmium in liver tissues (77.9 µg/g) followed by Rajanpur (44.9 µg/g), Muzaffar Garb (28.4 µg/g), Vehari (28.4 µg/g) and Khanewal (28.6 µg/g) has the lowest value of cadmium in the liver. The range of Cd in the liver is (9.43-83.11) and mean concentration of Cd in liver is (44.41 µg/g). It is at high toxic level limits at all study sites.

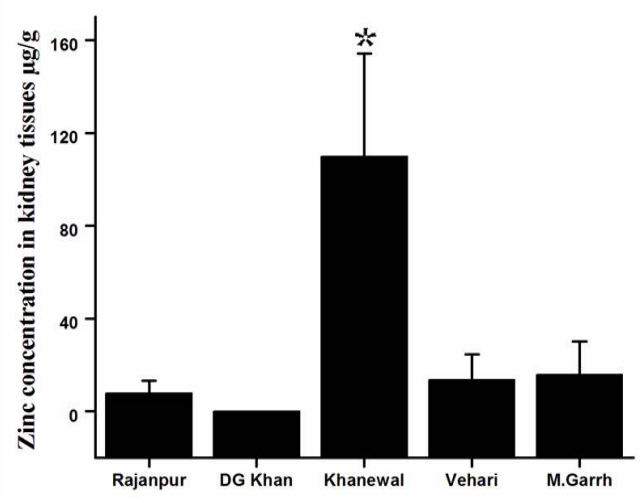


Figure 4. **Comparison between mean concentration of zinc in liver tissues (n=20) of house crow (*Corvus splendens*) from southern Punjab, Pakistan.**
Khanewal has the highest mean value of Zn in kidney yissues 109.9 µg/g followed by Muzaffar Garb (15.8 µg/g), Vehari (13.7 µg/g), Rajanpur (7.9 µg/g) and the lowest value is found in Dera Ghazi Khan (0 µg/g)_ The range of zinc in the kidney is (0.22-170.28 µg/g) and mean concentration of zinc in the kidney is (29.46 µg/g) _ Thereby the zinc concentration in kidney and liver is below toxic level at all study sites.

Table 3. Mean concentration of metals in Kidney samples of House Crow (*Corvus splendens*) from urban areas of five districts of Southern Punjab, Pakistan. Given are means± SE (Geometric means are given below in parentheses). Comparison is made with one-way ANOV A. All values are in ppm wet weight.

Location	Zn(µg/g)	Cu (µg/g)	Cd (µg/g)
RAJ	7.9±5.25 (5.9)	2.27± 1.27 (1.59)	46.1±12.5 (41.98)
DGK	0±0 (0)	3.35±0(3.35)	90.04±0 (90.04)
KWL	109.9±44.4 (79.46)	7.69±3.55 (6.22)	11.87± 1.11 (11.76)
VHR	13.7± 10.9 (8.32)	3.71±0.828 (3.54)	15.39±1.33 (15.27)
M.G	15.8± 14.3 (3.71)	8.64± 1.97 (8.41)	40.9±20.3 (20.35)
ANOVA F (*P*)	2.78 (0.175)	1.79 (0.236)	3.62 (0.057)
Toxic level	> 200	> 1	> 3

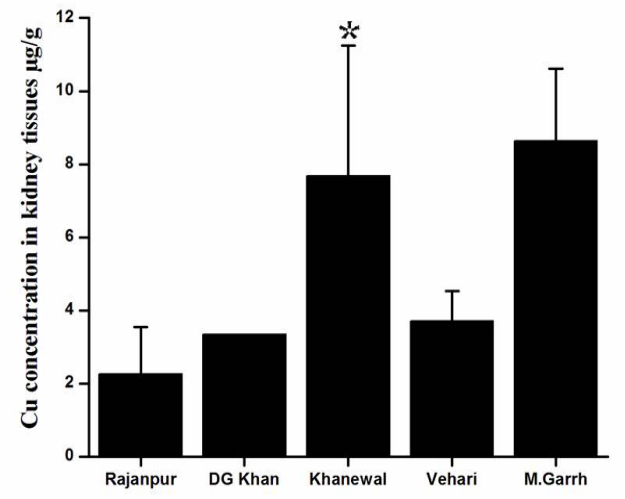


Figure 5. **Comparison between mean concentration of copper in liver tissues (n=20) of house crow (*Corvus splendens*) from southern Punjab, Pakistan.**
Muzaffar Garb has the highest mean value of Cu in kidney tissues (8.64 µg/g) followed by Khanewal is (7.69 µg/g), Vehari (3.71 µg/g), Dera Ghazi Khan (3.35 µg/g) and the lowest value of copper found in Rajanpur (2.27 µg/g). The range of copper in kidney is 0.37-28.75 µg/g and mean concentration of copper in kidney is (5.13 µg/g).The values is above toxic level at all study sites.

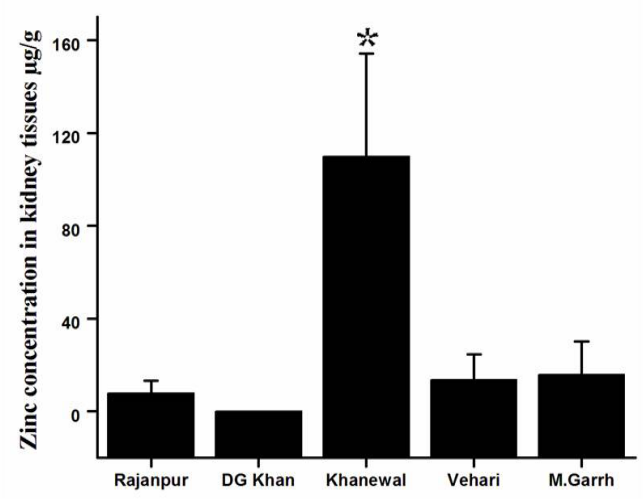


Figure 6. **Comparison between mean concentration of cadmium in liver tissues (n=20) of house crow (*Corvus splendens*) from southern Punjab, Pakistan.**
Dera Ghazi Khan has the highest mean value of cadmium in kidney tissues (90.04 µg/g) followed by Rajanpur (46.1 µg/g), Muzaffar Garb (40.9 µg/g), Vehari (15.39 µg/g) and the lowest value of cadmium in Khanewal (11.8 µg/g)_ The range of cadmium in kidney is (2.41-109.86 µg/g) and mean concentration of cadmium in kidney is (40.86 µg/g).

4. Discussion

The objective of this study was to find out metal level among different study sites. There was significant variation in metals among study sites but none of the single sites is designated as the highest or lowest level for all elements. The same site has the highest value of one element and lowest of others. For example house crow samples of Khanewal have the highest mean concentration of zinc (109.9 µg/g) and lowest mean concentration of cadmium (28.67µg/g).

There is no published data available for metals study particularly in house crow (*Corvus splendens*) a scavenger species. The data was compared with other raptors. Additionally, most of the published data is available on metal in kidney and liver related to seabirds, waders studies and a very few data available on birds of prey.

In four species of diving ducks liver Zn concentration in the range of 242-548 µg/g (normal value is 35.9 µg/g wt. weght)[37]. Its concentration in the liver of Wild Birds in the Tri-State Mining District in the range of 86-440 µg/g (Beyer *et al.*, 2004) while[38] found liver Zn concentration in Black kite (*Milvusmigrans*) is 63.68 µg/g. Mean Zn concentration in liver samples of some wild birds from Korea in the range of 6.53 µg/g-126 µg/g[39].

According to our study house crow samples of Khanewal has highest mean value of zinc in liver is 80.2 µg/g followed by Rajanpur (22.7 µg/g), Dera Ghazi Khan (20.56 µg/g), Vehari (18.3 µg/g) and the lowest value is found in the samples of Muzaffar Garh (15.6 µg/g). The range of Zn in the liver is (1.38-132.8 µg/g) and the mean concentration of zinc in the liver is (31.53 µg/g). Our data is closely related to the work of[40].

Zinc concentrations in the kidney of Mallard ducks range between 27-519 ppm[41], while[38] found kidney zinc concentration in Black kite (Milvus migrans) as 56.33 µg/g. The range of mean concentration of zinc in kidney tissues of raptor species is 14.92–100.2 ppm (d.w), Toxicity level of zinc in birds is >200 ppm[42]. Zinc in the kidney of

graylag geese (189.2 µg/g), red knots (104.6 µg/g) and in grey plovers is (100.3 µg/g)[43].

In our study house crow samples of Khanewal has the highest mean value of Zn in the kidney is (109.9 µg/g) followed by Muzaffar Garh samples (15.8 µg/g), samples of Vehari (13.7 µg/g), Rajanpur samples (7.9 µg/g) and the lowest value is found in the samples of Dera Ghazi Khan (0 µg/g). The range of zinc in the kidney samples is (0.22-170.28 µg/g) and the mean concentration of zinc in the kidney samples is (29.46 µg/g). Zinc concentration in kidney and liver is below the toxic level at all study sites. Our data is closely related to the work of[43].

Cadmium concentration in birds was found in higher level in kidneys, lower level in liver and found in very low concentration in muscle. Cadmium concentration above 3 mg/kg dry weight in liver samples and above 8 mg/kg dry weight in kidney samples indicated an increased environmental exposure[44]. Cadmium concentration found in samples in the range of (0–3 mg/kg) is considered as normal level, (4–8 mg/kg) considered as higher level and (49 mg/kg) considered as toxic level in wet weight liver samples from the closely related white-tailed ptarmigan45. Threshold concentrations of Cd poisoning in birds would be expected at 40 mg/kg wet weight (approximately 130 dw) in liver and at 100 mg/kg wet weight (approximately 330 d.w) in the kidney[40].

In peregrine falcon in Sweden the concentration of Cd in liver and kidney samples is 3 µg/g and 2–8 µg/g respectively (Kristine *et al.*, 2004). Cd level in the liver of ruffed grouse in North America between 3.9-49 mg/kg in wet weight[46] and in willow ptarmigan in Northern Quebec 179.7 µg/g (d.w) in kidney tissues and 25.8 µg/g (d.w) in liver tissues[47]. Cd in the adult scups (Aythya marila) in liver 2.59 mg/kg and in kidney 10.31 mg/kg (d.w)[48] and for common eider ducks that are found in Canadian arctic the cadmium concentrations in kidney ranged from (47–281 µg/g d.w) while in liver its value is from 0.11–1.2 µg/g[49].

Cd level in White-Tailed Eagles was found in the range of liver tissues (0.004–0.663 ppm) and in kidney tissues (0.011–0.665 ppm)[50] and [38] studied cadmium level in Black Kite (Milvus migrans) from Southern Punjab Pakistan and concluded that Cd concentration in kidney is (2-4 µg/g) and in liver (1-2 µg/g).

According to our study house crow samples of Dera Ghazi Khan has a highest mean value of cadmium in kidney (90.04 µg/g) followed by Rajanpur samples (46.1 µg/g), samples of Muzaffar Garh (40.9 µg/g), Vehari samples (15.39 µg/g) and the lowest value of cadmium is found in the samples of Khanewal district (11.87 µg/g). The range of cadmium in kidney samples is (2.41-109.86 µg/g) and the mean concentration of cadmium in kidney samples is (40.86 µg/g). Our results show high values of cadmium in kidney as compared to the above mentioned studies.

In northern gos hawks Cd concentrations found in a liver sample is 0.06 µg/g in wet weight[50].

According to our study house crow of Dera Ghazi Khan has the highest mean value of cadmium in liver (77.9 µg/g) followed by Rajanpur samples (44.9 µg/g), Muzaffar Garh samples (42.2 µg/g), Vehari samples (28.42 µg/g) and samples of Khanewal has lowest value of cadmium in the liver. Cd range in the liver is (9.43-83.11) and means the concentration is (44.41µg/g). Our results show high values of Cd in liver as compared to above mentioned studies. When we compare mean cadmium concentration in kidney and liver, it is at high toxic level limits at all study sites. A high level of Cd indicates the pollution in the environment at all study sites due to more urbanization and heavy burning of fuels.

Cu level in seabirds from Gough Island in the kidney is 8 µg/g wet weights and in liver is 3µg/g[51]. Mean concentrations of Cu in Pelagic seabirds and argued that Cu concentration in kidney is 4.71 µg/g and liver 5.93 µg/g[52]. Cu concentration in kidney is 12-21 µg/g in the three bird species of polluted wetland[53]. Cu concentration was found in kidney samples of Great Tit is 16.11 µg/g and in liver is 37.47 µg/g, Greenfinch at the western mountain of Beijing was 20.62 µg/g in kidney samples and 27.01 µg/g in liver samples[54].

In our study house crow of Muzaffar Garh has a highest mean value of Cu in the kidney is (8.64 µg/g) followed by Khanewal samples (7.69 µg/g), Vehari samples (3.71 µg/g), Dera Ghazi Khan samples (3.35 µg/g) and lowest value of copper is found in house crow samples of Rajanpur (2.27 µg/g). The range of copper in kidney was 0.37-28.75 µg/g and the means concentration was (5.13 µg/g).

Range of copper in liver of six waterfowl species at Donana Natural Park is 7.30-742.96 mg/kg (Taggart *et al.*, 2006). Mean average concentration of copper in the liver of fledgling of black headed gulls in the dam reservoir in south-western Poland is 36.72 µg/g dry weights[55].

House crow samples of Muzaffar Garh has a highest mean value of copper in liver (12.78 µg/g) followed by Vehari samples (7.39 µg/g), Khanewal samples (7.26 µg/g), Dera Ghazi Khan samples (4.13 µg/g) and the lowest value of

copper found in house crow samples of Rajanpur (3.67 µg/g). The range of copper in liver is (0.39-21.35 µg/g) and the means concentration of copper in liver is 7.04 µg/g which clearly indicates the copper concentration above the permissible limits and pose a health risk to biological species with particular reference to house crow (*Corvus splendens*)

5. Conclusion

It is concluded from the above study that higher industrial rate in urban areas of Pakistan lead to heavy metal toxicity which is a major problem that is associated with severe health issues. This study related to find out the concentration of different metals in different areas that affects on living organisms especially humans.

6. Acknowledgement

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7. Conflict of Interest

Authors declared no conflict of interest.

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