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Heavy Metals Mediated Genotoxic Effects on Captive *Pavo cristatus* from Different Sites of the Punjab, Pakistan

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ABSTRACT

Heavy metal pollution is a big problem not only for humans but also for captive animals. The current study was conducted to monitor the heavy metal pollution in a non-invasive way and to investigate the DNA damage, particularly due to heavy metals contamination in the environment of captive Indian Peafowl (*Pavo cristatus)* kept at different geographical regions of Punjab (Jallo Wildlife Park Lahore, Wildlife Park Bahawalnagar and Wildlife Park Murree). For that purpose, Surface soil, deep soil, water, fecal and feed samples were collected as indicators to estimate the heavy metals pollution in the environment of captive *P. cristatus*. Cr. Pb, Ni, Co and Mn were identified through atomic absorption spectrophotometer. The highest accumulation of heavy metals was found in feed samples followed by fecal matter as compared to water and soil samples in all the sites. Cr was found highly abundant in all sites than other metals. The highest heavy metal pollution was observed in Wildlife Park Murree followed by Jallo Wildlife Park Lahore and least in Wildlife Park Bahawalnagar. Blood samples of *P. cristatus* were collected from all sites to assess the DNA damage by single-cell gel electrophoresis (comet) assay. *P. cristatus* kept at Wildlife Park Murree and Jallo Wildlife Park Lahore had almost same and greater DNA damage than the birds kept at Wildlife Park Bahawalnagar. Heavy metal concentrations and variation in DNA damage were correlated. Only Cr and Mn concentrations were directly associated with the LHead and LComet variations. This study concluded that heavy metals contamination exposure could be detected by a no-invasive way and Wildlife Park Bahawalnagar is most suitable for captivity with the least heavy metal contamination and genotoxicants.

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Key words
Indian peafowl, Captivity, Heavy metals pollution, Atomic absorption spectrometry, Genotoxicity, DNA damage

INTRODUCTION

Wildlife has been declined from last decade globally from their wild habitat. This situation is increasing in Pakistan alarmingly. In Pakistan, Indian peafowl (*Pavo cristatus*) has become locally extinct from some areas of its past distribution range and rare in the wilderness due to numerous threats to its existing population. They serve a significant role in an ecosystem as a useful indicator of the environmental quality around them. Their attractive long train feathers make them prominent to predators and humans. The main reasons for its extirpation in Pakistan are habitat loss and degradation, illegal poaching, human population pressure, intensive agricultural practices and use of pesticides, killing for medicinal purposes, trapping and collection of eggs for consumption (Anwar *et al*., 2015; Naz *et al*., 2020; Jose and Nameer, 2020). Therefore, the killing of *P. cristatus* especially for hunting is prohibited under the Punjab Wildlife Act 1974 (Protection, Preservation, Conservation, and Management) (Mushtaq-ul-Hassan *et al*., 2012).

A few decades ago, the Punjab Wildlife Department prioritized captive breeding of *P. cristatus* on a commercial basis to make Phasianidae as street birds (Maan *et al*., 2016; Mushtaq-ul-Hassan *et al*., 2012). Captivity helps to prevent it from being endangered (El-Shahawy and Ismail, 2010). In captivity, *P. cristatus* can live for about 23 years but 15 years in the wild (Flower, 1938). Although captivity helps to conserve *P. cristatus*, it also has some consequences. The confined space puts the birds under potential stress which can lead to lowered performance and malnutrition (Naz *et al*., 2020; Tanveer *et al*., 2021). Initially, most of the zoos or Parks were located away from the cities. Due to outgrown urbanization, the majority of the zoos are now surrounded by human residences. So, along with the captivity stress, they also face environmental pollution that is increasing progressively (Gupta and Bakre, 2013).

Environmental pollution has become one of the major

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problems not only for humans but also for lives under captivity (Naz *et al*., 2021).

Heavy metals are being continually released into environment by different geological and anthropogenic activities. i.e., volcanic activity, mining or weathering of rocks, coal combustion, industrial activities and some agricultural processes. Therefore, the environment is currently being exposed to the greatest level of heavy metals within recorded history and it has become a major problem in recent years (Agarwal, 2002; Wagner and Boman, 2003; Rezania *et al*., 2016; Asaduzzaman *et al*., 2017). These are highly persistent in the environment because these are non-thermo-degradable and non-biodegradable.

Heavy metals have many adverse health effects including genotoxicity. The most common and deleterious effects are DNA lesions specifically DNA breaks (García-Lestón *et al*., 2010). DNA strand breaks further lead to disturbing the configuration and integrity of nucleic acid. Ultimately, these breaks trigger the death of the cell. Furthermore, free radicals are produced by the toxicity of heavy metals or from other ways. These radicals target the DNA which contains all genetic information and eventually alter the transcription of genes. Hence, their effects lead to the point of protein synthesis (Yuan and Tang, 2001).

Some other toxic effects of heavy metals in birds may result in low reproductive rate, thinning of eggshell, reduction of growth rate, impairment of immune system, developmental deformities and malformations. As a result, the population of birds starts declining (Dauwe *et al*., 2006). It is therefore the need of time to assess and monitor their existence, evaluate their amount, manage and remediate biological and ecological damage (Movalli, 2000; Naccari *et al*., 2009). Moreover, It was to be verified that whether the stresses of captivity along with the heavy metal pollution can impair the DNA of *P. cristatus* or not. In bird species, the effects of heavy metals have been studied in terms of their bioaccumulation and toxicity. But not much is known about genotoxicity, specifically under captive conditions. Thus keeping in view the different factors, this study was aimed to compare the heavy metals' contamination and its genotoxic effects in captive *P. cristatus* kept at different regions of Punjab, Pakistan.

MATERIALS AND METHODS

Study area

Three Punjab Wildlife Parks, i.e. Jallo Wildlife Park Lahore, Wildlife Park Bahawalnagar and Wildlife Park Murree were selected as study sites. These sites are located in three different geographic areas according to Koppen Climate Classification reported by Sarfaraz *et al*. (2014) and have different management conditions and environments. *P. cristatus* has a great ability to adapt to wide-ranging habitats with different environments. So, it was easily available in these distinct parks of Punjab, Pakistan.

Collection of samples

Fecal matter, water soil, feed and blood samples were used for determination of heavy metals. Freshly egested fecal samples of *P. cristatus* (10 samples from each site) were collected from the ground of their cages. To prevent contamination by stones, dust, or other particles, the samples were collected carefully and kept in a handling bag with ice packs (Gupta and Bakre, 2013). Water samples were collected in sterilized bottle and closed tightly. Those were taken from the tap of cages from all sites with proper labeling (Yasmeen *et al*., 2020). Soil samples were collected from 6 inch depth in zip lock bags from all the cages of the captive *P. cristatus* from all selected sites and appropriately labeled (Yasmeen *et al*., 2020). Samples of artificially prepared feed of *P. cristatus* were collected from feeding cups in cages. Ten blood samples (2-3ml) from each site were taken from brachial vein of each bird into the EDTA vacutainers (Samour *et al*., 2010).

Analysis of heavy metals

All samples of fecal, feed, water, and soil were digested before the analysis of heavy metals. A standard solution of conc. perchloric acid and nitric acid were formed in a 1:3 ratio. All the samples except water were oven-dried and 1g of dried samples in case of fecal, feed and soil were added to a standard solution. In the case of the water sample, 1ml of water was directly added to the standard solution of acids. Then it was heated at a temperature of 150 °C on a hot plate for 30 min. Then it was increased to 180 °C till the clear solution was formed. Then dilution of the cleared solution was done by using 20 ml distilled water and two drops of hydrogen peroxide. For the removal of residues, filtration of diluted sollution was done by Whatman filter paper. The filtered solution were kept in glass tubes which was already sterilized properly. All the samples were labeled properly and stored at room temperature till further analysis (Yasmeen *et al*., 2020; Gupta and Bakre, 2013; Sidra *et al*., 2019). The samples were chemically analyzed for heavy metals by running through Atomic Absorption Spectrometry (AAS) and different levels Cr, Pb, Ni, Co, and Mn were determined at their respective wavelength according to Yasmeen *et al*. (2020).

Analysis of DNA damage

Single cell gel electrophoresis (SCGE) also known as comet assay was performed to analyse the DNA damage induced by heavy metal contamination in peripheral blood erythrocytes of *P. cristatus*. It was performed by following the protocols suggested by Singh *et al*. (1988) and Tice *et al*. (2000) (Fig. 1). Seven parameters of comet assay, i.e. Length of head (L head), length of tail (L tail), length of comet (L comet), tail DNA, head DNA, tail length (L tail), head length (L head), comet length (L comet), Tail moment (TM) and olive tail moment (OTM) were analyzed and recorded by Casp_1.2.3b1 computer software.

Statistical analysis

All statistical analysis of data was done by IBM SPSS Statistics 21 software. The correlation between the heavy metal concentrations and variations in DNA damage was assessed. One way ANOVA (Analysis of variance) was applied to compare heavy metals concentrations and variations in DNA damage among all selected sites (Steel *et al*. 1997). Means were also compared by using Tukey's range test.

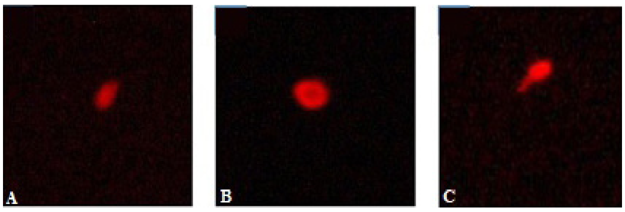

Fig. 1. Microscopic images of blood samples of captive *P. cristatus* after comet assay (A) kept at Jallo Wildlife Park Lahore (B) Kept at Wildlife Park Bahawalnagar (C) kept at Wildlife Park Murree.

RESULTS

The significant differences ($p<0.05$) were observed among mean concentrations of all metals in various types of samples from different localities. By comparison of surface soil of all three sites, Cr, Pb, Co and Mn were found highly abundant in samples of Murree. While Ni was found higher in Bahawalnagar. On the other hand, the lowest concentration of Pb, Ni, Co in the surface soil of Lahore and Cr, Mn in the surface soil of Bahawalnagar was observed. It was observed that Cr, Pb, Ni and Mn were highly abundant in the deep soil of Murree. While Co was found higher in Bahawalnagar. On the other hand, the lowest concentration of Pb, Ni, Co and Mn in the deep soil of Lahore and Cr in the deep soil of Bahawalnagar was observed. The concentration of Pb and Mn were greater in the water of Murree. While Cr and Ni were found higher in Lahore and Co was abundant in the water of Bahawalnagar. On the other hand, the lowest concentration of Pb, Co and Mn in the water of Lahore and Cr, Ni in the water of Bahawalnagar was observed.

Table I. Accumulation of heavy metals (Mean ± S.D) in fecal, water, feed and soil samples from Jallo Wildlife Park Lahore, Wildlife Park Bahawalnagar and Wildlife Park Murree.

Metals	Jallo wildlife park Lahore	Wildlife park Bahawalnagar	Wildlife park Murree
Cr (µg/g)			
Surface soil	6.775 ± 0.05^{b}	1.521 ± 0.72^{c}	8.1 ± 0.065^{a}
Deep soil	3.1 ± 0.042^{b}	2.36 ± 1.708^{b}	32.07 ± 0.053^{a}
Water	12.10 ± 0.081^{a}	2.025 ± 0.167^{c}	7.80 ± 0.042^{b}
Feces	5.57 ± 0.045^{b}	1.575 ± 0.045^{c}	33.85 ± 0.061^{a}
Feed	31.325 ± 0.042^{b}	0.275 ± 0.057^{c}	34.25 ± 0.387^{a}
Pb (µg/g)			
Surface soil	0.275 ± 0.047^{b}	0.387 ±0.093^{a}	0.40 ± 0.063^{a}
Deep soil	0.25 ± 0.042^{b}	0.419 ± 0.156^{a}	0.45 ± 0.035^{a}
Water	0.225 ± 0.042^{c}	0.362 ± 0.08^{b}	0.475 ± 0.035^{a}
Feces	0.30 ± 0.039^{b}	0.225 ± 0.045^{c}	0.675 ± 0.039^{a}
Feed	0.525 ± 0.0387^{c}	0.60 ± 0.052^{b}	0.850 ± 0.042^{a}
Ni (µg/g)			
Surface soil	0.05 ± 0.022^{b}	0.332 ± 0.094^{a}	0.325 ± 0.044^{a}
Deep soil	0.025 ± 0.004^{c}	0.236 ± 0.075^{b}	0.35 ± 0.045^{a}
Water	0.350 ± 0.035^{a}	0.187 ± 0.146^{b}	0.275 ± 0.05^{ab}
Feces	0.10 ± 0.035^{b}	0.275 ± 0.047^{a}	0.225 ± 0.039^{a}
Feed	0.575 ± 0.035^{a}	0.125 ± 0.045^{c}	0.350 ± 0.039^{b}
Co (µg/g)			
Surface soil	0.125 ± 0.039^{a}	0.144 ± 0.1^{a}	0.150 ± 0.032^{a}
Deep soil	0.10 ± 0.042^{a}	0.281 ± 0.201^{a}	0.225 ± 0.093^{a}
Water	0.10 ± 0.5^{b}	1.375 ±0.471^{a}	0.20 ± 0.047^{b}
Feces	0.175 ± 0.042^{b}	0.175 ± 0.057^{b}	0.450 ± 0.052^{a}
Feed	0.225 ± 0.047^{c}	0.375 ± 0.042^{b}	0.454 ± 0.062^{a}
Mn (µg/g)			
Surface soil	0.425 ± 0.042^{a}	0.406 ± 0.055^{a}	0.925 ± 0.057^{b}
Deep soil	0.35 ± 0.042^{a}	0.431 ± 0.059^{b}	0.70 ± 0.045^{c}
Water	0.30 ± 0.042^{a}	0.375 ± 0.185^{a}	0.60 ± 0.055^{b}
Feces	0.275 ± 0.022^{a}	0.30 ± 0.035^{a}	0.70 ± 0.045^{b}
Feed	0.775 ± 0.05^{a}	0.375 ± 0.039^{b}	0.90 ± 0.039^{c}

Mean value bearing different superscripts ($^{a, b, c}$) in a row are statistically significant.

All selected metals were found higher in fecal samples of Murree except Mn. Mn was significantly higher in feces from Bahawalnagar. Contrastingly, the lowest concentration of Cr, Pb, and Co in fecal samples from Bahawalnagar, while Ni and Mn were lowest in fecal samples from Lahore. It was detected that Cr, Pb, Co and Mn were significantly higher in feed samples from Murree. Whereas, Ni was found higher in feed samples from Lahore. Moreover, Cr, Ni and Mn were found lowest in feed samples of Bahawalnagar while the Pd and Co were in feed samples of Lahore. Murree was more polluted with all selected heavy metals except Co. Co contamination was higher in Lahore. Among all detected heavy metals, Cr contamination was significantly higher in all sites followed by Mn, Pb, Ni and Co (Table I).

Analysis of variance was conducted for each comet parameter among all three selected sites. It showed that mean values of L head, L tail, L comet, head DNA, tail DNA were significantly different at a level of $P<0.05$. While TM and OTM values were observed similar at all sites with a negligible amount. The variation pattern in LComet was in the manner of Murree = Lahore > Bahawalnagar. Between group comparison by Post Hoc Tukey test showed that DNA variations in *P. cristatus* kept at Wildlife Park Murree and those who kept at Jallo Wildlife Park Lahore were almost similar. But, the variations in DNA of *P. cristatus* kept at Wildlife Park Bahawalnagar were different from comparative ones (Table II). A significant difference was also observed during microscopy as demonstrated by Figure 1.

Table II. Different comet parameters (Mean±SD) in blood of captive *P. cristatus* kept at Jallo Wildlife Park Lahore, Wildlife Park Bahawalnagar and Wildlife Park Murree.

Factors	Lahore	Bahawalnagar	Murree
L Head	22.20 ± 4.607[a]	16.36 ± 4.99[b]	23.88 ± 17.668[a]
L Tail	4.96 ± 3.01[a]	3.28 ± 1.05[b]	4.44 ± 2.177[a]
L Comet	27.16 ± 6.04[a]	19.64 ± 4.89[b]	28.32 ± 7.791[a]
Head DNA	94.79 ± 7.78[a]	92.53 ± 20.35[b]	87.36 ± 11.91[ab]
Tail DNA	5.21 ± 7.78[b]	7.47 ± 11.91[ab]	12.64 ± 20.35[a]
TM	0.408 ± 0.846[a]	0.343 ± 1.022[a]	0.756 ± 1.433[a]
OTM	0.64 ± 1.166[a]	0.491 ± 0.754[a]	0.798 ± 1.372[a]

Mean values bearing similar superscripts ([a,b,c]) in a row are statistically non-significant.

The correlation of each comet parameter with each heavy metal abundance showed that only Cr and Mn concentrations were associated positively or directly with the L head and L comet variations. This means that L head and L comet values will be increased by increasing the exposure of Cr and Mn around *P. cristatus*. All other parameters have a very weak and no association with the exposure of detected metals (Table III).

DISCUSSION

Environmental pollution has become a big problem and increased day by day. Organic pollutants are biodegradable but inorganic like heavy metals can never be converted into less toxic form (Gupta *et al*., 2003). As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn are a group of toxic metal pollutants reported in Pakistan (Shakir *et al*., 2016). In the current study, Cr, Pb, Ni, Co and Mn were detected. Concentrations of these metals were detected at varying levels. Levels of these metals in fecal and feed samples were detected higher than the level in water and soil samples, which was an indication of getting exposed to the body of captive *P. cristatus* with heavy metal pollution. A similar fact was observed by Sidra *et al*. (2019) in different captive animals including mammals, reptiles and birds. All metals were found highest in feed samples from all sites and reflecting in fecal matter. A similar fact was observed by Yasmeen *et al*. (2018) when they detected Ni in the fecal and feed samples of the African lion. But, Leonzio and Massi (1989) reported that metal concentration in feed is usually equaled to the concentration detected in fecal matter. So, keeping in mind this fact, the current study reveals that higher concentration in the feed may lead to bio-accumulate into the body. Different studies described and agreed that a significant concentration of metals in fecal excreted when organisms were exposed to those metals (Nigra *et al*., 2016; Yabe *et al*., 2018; Li *et al*., 2019).

Table III. Correlation analysis between comet parameters and different heavy metals exposure to captive *P.cristatus* kept at three distinct sites of Punjab, Pakistan.

	Cr	Pb	Ni	Co	Mn
L Head	0.4**	0.05	0.1	0.1	0.3**
L Tail	0.1	-0.05	0.1	0.1	0.03
L Comet	0.4**	0.03	0.1	0.1	0.3**
Head DNA	-0.1	-0.1	-0.1	0.03	-0.04
Tail DNA	0.1	0.1	0.1	-0.03	0.04
TM	0.1	0.02	0.1	-0.02	0.04
OTM	0.1	0.01	0.1	-0.02	0.00

**correlation is significant at 0.01 level.

Cr is mostly found in the earth crust and water. Cr is important for living bodies to metabolize carbohydrates. But, excess Cr causes serious health problems like cancer, skin diseases, hematological impairment and oxidative stress. Major Cr contamination source is the wastewater from industries (Cefalu and Hu, 2004; Shakir *et al*., 2016). Cr concentration was highly significant in all types of samples in each site. Similar findings were reported by (Perugini *et al*., 2011). They used honey bees as bioindicator to find out the heavy metals contamination in wildlife reserves at Italy. Contrastingly, they found no significant different concentrations among different sampling sites. But in the current study, the concentration of Cr is significantly different in different sites of Punjab, Pakistan. The highest Cr concentration was detected in Wildlife Park Murree, followed by Jallo Wildlife Park Lahore and the least concentration of Cr was observed at Wildlife Park Bahawalnagar. The concentration of Cr in a specific time and area depends on the industrial process and released amount of Cr. Then the atmospheric Cr depends on the meteorological factors for covering a long distances.

Pb is basically one of the major environmental pollutants that is dangerous for health also. It is highly toxic to the liver, kidney and nervous system. Its main sources include traffic, combustion, steel factories, cement industry, waste disposal, forest fire and volcanic activities. It deposits into the soil from the air. Soil contains organic matter which makes it immobilized and its exposure to living one's increases by deposition. Its major exposure pathway is ingestion through feed and water. Soft water has the capacity to dissolve Pb. Drinking water can be contaminated with Pd by different types of transporting pipes. Pb concentration in soil samples 7.70 mg/Kg was reported by Pandey (2012). This concentration was much higher than that of all selected sites of our study. For this, the most possible reason was found a settled dust. Pd concentration was found highest at Wildlife Park Murree, Followed by Wildlife Park Bahawalnagar and least was at Wildlife Park Lahore. Overall, each sampling site had a much greater concentration of Pd than the WHO recommended limit (0.1 ppm) of Pb in the human body. However, considering the body size, human beings are big sized organisms as compared to *P. cristatus* in the current study, it can be estimated that the detected level of Pb in our study can evidence to be more toxic due to the smaller body size of the studied bird *P. cristatus*. The amount of Pb was higher in all our study sites as compared to the endurable limit (0.1 mg/Kg) reported by (Nkansah and Ansah, 2014). The major source of Pb is vehicular smoke.

In contrastingly, (Gupta and Bakre, 2013) reported much higher concentrations of Pd and much lower concentrations of Cr in lions fecal samples of Jaipur zoo than our findings in each site. They reported Pb and Cr were 56.0 mg/kg, 3.19 mg/kg, respectively. Its most probable reason might be specie difference and more industrialized area.

Ni, Co and Mn are also known as essential micronutrients (Reeves and Baker, 2000). Ni plays an important role in the good health of animals as it is a component of a urease enzyme (Nagajyoti *et al*., 2010). Exposure of Ni with high concentration can cause deleterious health issues like respiratory diseases, cancer and kidney problems (McGregor *et al*., 2000; Wadhwa *et al*., 2015; Shakir *et al*., 2016). Ni is emitted into our environment through weathering, combustion, forest firing, sewage sludge and incineration of waste (Genchi *et al*., 2020). Ni concentration was found highest at Wildlife Park Murree followed by Wildlife Park Bahawalnagar and Jallo Wildlife Park Lahore. Pb, Cr and Ni concentration was detected higher in soil samples as compared to water samples of Wildlife Park Bahawalnagar and Wildlife Park Murree. A similar fact was reported by Yasmeen *et al*. (2018). In contrast to their study, the water of Lahore was more contaminated with these metals as compared to soil. The only reason for its contamination might be from the transporting pipes connecting to sewage sludge.

Co is essential for many enzymatic activities and the synthesis of B_{12} in the body (Gal *et al*., 2008). High exposure to Co leads to respiratory disorders and cancer (Shakir *et al*., 2016). Co is released into the environment through mining activities and coal combustion. The highest concentration of Co in Soil was reported by (Khan *et al*., 2008) in KPK. Murree is more closely located with KPK as compared to Lahore and Bahawalnagar. But in the current study, the highest concentration of Co was detected in the soil of Bahawalnagar region, Punjab.

Mn is naturally found in the environment (soil, water and air). It is majorly found in the earth crust. It is an essential element for many enzyme activities in the body (Affum *et al*., 2011). Its high concentration causes neurological disorders, anemia and oxidative stress. Exposure of Mn is through a number of activities like mining, sewage sludge, wastewater discharge and mineral processing (Andrade *et al*., 2015; Shakir *et al*., 2016). Alam *et al*. (2011) reported that Mn was found above the permissible limit in Rawalpindi and Lahore. Similar results were found in the current study as we found the highest concentration of Mn at Wildlife Park Murree. Which is located under the district of Rawalpindi.

In the current study, Jallo Wildlife Park Lahore has more heavy metal exposure as compared to Wildlife Park Bahawalnagar. Similar findings were reported by Yasmeen *et al*. (2020). According to them, Lahore is more polluted than Bahawalpur. Bahawalpur is located just aside from Bahawalnagar in a same climatic zone. So, their findings are very close to our results. Our results also oppose the statement reported by Stone *et al*. (2010) that Lahore is considered an emerging megacity. It is heavily polluted with high levels of contaminants in the air, contributing to an extreme level of pollution. Our results indicated that Murree is a more polluted city with regard to heavy metal pollution in Punjab as compared to Lahore. The possible reason may be Jallo Wildlife Park Lahore is located in the periphery of polluted city Lahore.

Comet assay proved to be a sensitive and efficient technique in the assessment of DNA damage in birds and animals. The previously cited literature on comet assay enlightened the fact the animals were subjected to some exogenous source of DNA damage in order to estimate the amount of genetic damage. The relation between ecological tragedy and genotoxicity was measured. In the current study, the DNA damage was assessed in captive *P.cristatus* kept at Jallo Wildlife Park Lahore, Wildlife Park Bahawalnagar and Wildlife Park Murree. The birds have several challenges here but heavy metals exposure and captivity stress were focused in the present study.

Our results indicated that only Cr and Mn exposure have a positive moderate association with DNA damage in captive *P. cristatus.* As the exposure of these metals increases, the DNA comet length increases. Similarly, Pastor *et al*. (2001) investigated the effect of heavy metals in white storks (*Ciconia ciconia*) after a massive disaster of toxic acid leakage in Donana National Park. Measurable damage in the DNA was observed due to heavy metal exposure. But they observed that TM were higher in chicks present in the park. Contrastingly, in our study, there is no effect of any detected heavy metal with the TM. Its possible reason might be due to different heavy metals' groups.

According to the current study, Murree is more polluted with heavy metals as compared to other sampling sites. The DNA damage was also observed highest in the birds of Wildlife Park Murree. Bonisoli-Alquati *et al*. (2010) support these findings of the current study as they checked the DNA damage in blood cells of barn swallows (*Hirundo rustica*) in the Chernobyl region low-level radioactive contamination. They suggested that DNA damage in the birds of the contaminated areas was more as compared to the birds living in far off areas. This statement is supporting our findings.

A similar study was performed to check the effect of pollution on DNA damage in fishes by Gomes *et al.* (2018). They checked the genotoxicity in *Geophagus brasiliensis* fish in the Doce river basin. The disaster of tailings influx by a mining company took place in Mariana. The study was performed before and after the accident. Increased genetic damage was reported after the accident. However, the evidence of genetic damage in fish even before the accident was also reported. It was suggested that the presence of local industry near the lake margins and effluents discharge from it was responsible for this damage before the disaster took place. Those effluents discharge might have toxic heavy metals. Similarly, the effects of the discharge of effluents on DNA damage were also observed by Escobar (2015). Winter *et al*. (2004) also checked the DNA strand breaks in feral and caged *Leuciscus cephalus* fish exposed to polluted water. The DNA damage was observed in the form of long tail lengths.

Captive *P. cristatus* was subjected to captivity stress and several pollutants like heavy metals that could cause DNA damage. It was evident from the current study that DNA damage in captive *P. cristatus* was increased with the increased level of heavy metal exposure. As the highest level of heavy metals exposure was observed in Murree and the DNA damage was also observed greater in captive *P. cristatus* kept at Wildlife Park Murree.

All three selected sites are located in different climatic zone according to the Koppens climatic classification. All three parks belong to different geographic areas and environments. Jallo Wildlife Park Lahore has a steppe hot climate with dry winter. Wildlife Park Bahawalnagar has a hot climate with dry winter. While Wildlife Park Murree has a warm temperate climate without any dry (Sarfaraz *et al*., 2014). *P. cristatus* has a great ability to adapt to wide-ranging habitats with different environments. So, it was easily available in these distinct parks of Punjab, Pakistan. All three parks have different management conditions. Very good captivity management conditions at Jallo Wildlife Park Lahore were observed. There were many cages in which peafowls were kept properly for breeding purposes. That's why there was the highest number of *P. cristatus* (170). On the other hand Wildlife Park Murree has very poor management as compared to the other two parks. That's why it has the least number of captive *P. cristatus* (32). There was only one big cage in which all *P. cristatus* were kept and there was not any management for their breeding to increase their number. The Wildlife Park Bahawalnagar has only 38 captive *P.cristatus.* There were good management conditions, but Park management didn't have any good setup for their breeding.

CONCLUSIONS

This study concluded that Wildlife Park, Murree is more polluted with heavy metals while Wildlife Park Bahawalnagar is most suitable for captivity with the least heavy metal contamination. Cr was profoundly present in all captive sites of Punjab, Pakistan. There could be so many genotoxicants in the captive environment but our study revealed that Cr and Mn had a direct association with the DNA damage. *P. cristatus* kept at Wildlife Park Murree and Jallo Wildlife Park Lahore had more genotoxicity as they faced more pollution.

Proper management and control of these pollutants in the environment are required in order to protect this specie. Heavy metals studies should be carried out in other wildlife parks of Pakistan to evaluate the health status of captive animals and environmental quality. Other toxic metals should be detected in the environment of captive animals. Other genotoxicants should be identified.

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IRB approval and ethical statement

Members of the Institutional Review Board and Ethics Review Committee, Government College University, Faisalabad, Pakistan evaluated and approved this study.

Statement of conflict of interest

The authors have declared no conflict of interest.

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