



Short Communication

Faecal Matter of Spotted Deer (*Axis axis*) Acts as Bioindicator of Heavy Metals Contamination in the Air

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ABSTRACT

Heavy metals are high density compounds found in the earth crust and atmosphere. These metals in small amounts are important for normal metabolism of body but become very toxic when present in large amounts due to their capabilities of bio-accumulation in organisms. To see exposure of these metals in the environment, different invasive and non-invasive techniques are used. In the present study, enclosure of *Axis axis* commonly known as spotted deer was selected from Lahore and Bahawalpur zoo. Non-invasive technique was used to monitor heavy metal exposure and both biological (faecal, feed) and non-biological (water, soil) samples were collected. Soil was collected from different depths (6, 12, 18 inches) to study deposition patterns of metals. Samples were chemically digested and finally analysed by Flame Atomic Absorption Spectrometry (FAAS) for four heavy metals such as Pb, Cd, Zn and Cu. The results showed all the metals were in safe range in feed samples, a very negligible amount was present in water samples and small concentrations were noticed in soil samples. The amounts of Zn and Cu in feed were reflected in fecal samples; however, higher amount of Pb and Cd were noticed in fecal samples compared to feed, water and soil. This showed inhalation of these metals from the air. Amount of metals at different depths showed unique pattern low to high and again low that was irrespective of site location. Overall there was more exposure to air pollutants noticed for Lahore zoo compared to Bahawalpur zoo.

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Authors' Contribution

RY planned and supervised the study and wrote the manuscript. SS conducted the study. BNK co-supervised and planned the study. SSB and UR performed data analysis. AWQ reviewed the research.

Key words

Bio-accumulation, Invasive and non-invasive techniques, Biological samples, FAAS

Heavy metals such as arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), copper (Cu), and zinc (Zn) are high density compounds found in the earth crust and atmosphere (Duruibe *et al.*, 2007). These metals in small amounts are important for normal metabolism of bodies but become very toxic when present in large amounts due to their capabilities of bio-accumulation in organisms (Lenntech, 2012). The various studies described effect of heavy metals as these metals disturb the enzymes, interfere in metabolic processes such as synthesis of hemoglobin, effect on kidneys, gastrointestinal tract (GIT), joints and reproductive system, and can cause acute or chronic damage to nervous system and also responsible for mutagenesis which results in reduced fitness and increased health risks such as cancer and cardiovascular problems (Willer *et al.*, 2005; Yang and Sun, 2009; Davis, 2010; Praveena *et al.*, 2013). Urbanization is one of the most important factor that is polluting the environment by placing more and more concentrations of heavy metals (including essential ones) above their threshold levels.

Heavy metals usually enter the body through respiration, ingestion and skin and become poisonous only in specific forms such as Cr (III) is an essential trace element, while Cr (VI) exhibits the carcinogenic effects (Govind and Madhuri, 2014).

In many developing countries, environmental pollution including heavy metal contamination is a great concern (Cui *et al.*, 2009; Wei and Yang, 2010). Environmental degradation due to vehicular emitted heavy metals is affecting the soil particularly along road sides (De Silva *et al.*, 2016). Impact of pollution on soil degradation is also reported by Chao *et al.* (2014). Heavy metals can directly harm public health by entering the body with soil dust, dermal contact and breathing (Shi *et al.*, 2011). Different metals such as Cd, Pb, Zn, and Cu in soil can be transported through the food chain into the human body and thus are very toxic to people (Jarup, 2003; Ashish *et al.*, 2013; Gupta, 2013).

Heavy metals emissions from vehicles produced in tire wear, brake wear, and road abrasion as oil consumption is responsible for the largest emission for Cd, tire wear produced Zn, and brake wear is the most important source for Cu and Pb (Winther and Slento, 2010; De Silva *et al.*, 2016).

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Toxic effect of inorganic form of Pb in developing fetus and infant can cause disruption in biosynthesis of hemoglobin, anemia, high blood pressure, kidney damage, fertility problems and brain or nervous system damage (Ferner, 2001). The Cd derives its toxicological properties from its chemical similarity to Zn (an essential micronutrient for plants, animals and humans). Average daily intake of Cd for humans is 0.15 µg from air and 1 µg from water. Cd is a heavy metal that can cause kidney dysfunction if a person remained exposed for long time. Its high exposure may cause obstructive pulmonary disease and lung cancer. Bone defects (osteomalacia, osteoporosis) have also been reported in humans and animals by Satarug *et al.* (2003). Various toxic effects of Zn when this metal is present in excessive amount is also reported by (Nolan, 2003; Chen, 2011)

Environmental pollution is a big problem not only for humans but also for captive animals. To find the effect of air pollution on animals, various techniques are in use and reported in the studies (Beyer *et al.*, 2007; Roux and Marra, 2007; Džugan *et al.*, 2012). However, there is scarce of data regarding animals living in captive environment especially those zoological gardens which were built once in open areas but now surrounded by busy roads and high buildings.

In the present study feces of spotted deer (*Axis axis*) along other samples were used to evaluate heavy metals exposure in the captive environment.

Materials and methods

The enclosure of *Axis axis* commonly known as spotted deer or Chital was selected from Lahore and Bahawalpur Zoos. The enclosures of spotted deer in both zoos were located near the roads. The enclosure size of spotted deer covered an area of 1000 square foot with grass growing naturally in Bahawalpur zoo while 1106 square foot for Lahore zoo. There were 37 spotted deer (*Axis axis*) in Bahawalpur zoo and 8 spotted deer were found in Lahore zoo. Samples of faecal matter, feed, water and soil were collected in sterilized tightly closed bottle from both the zoos and properly labeled.

For detection of heavy metals the faecal, soil, feed and water samples were digested prior to heavy metal analysis. A stock solution of concentrated nitric acid and perchloric acid was prepared in 3:1 ratio. Samples were oven dried and one gm of dried samples were weighed and added to stock solution. The samples were heated on hot plate at temperature of 150 °C for thirty minutes. Then the temperature was increased upto 180 °C till the end point was attained in the form of clear solution. The digested solution was then diluted with double distilled water up to 20 ml of and 2 drops of hydrogen peroxide was added.

The solution was filtered by using Whatman filter paper to remove the residues. Water samples were digested with analytical grade HNO₃. The sample were stored in sterilized Borosil glass tubes and kept at room temperature prior to analysis (Gupta, 2013). The chemical analysis of heavy metals was carried out with the help of Flame Atomic Absorption Spectrometry and levels of lead (Pb), Copper (Cu), Cadmium (Cd) and Zinc (Zn) were determined at respective wavelength of 217, 324.7, 228.9, and 213.9 nm (Perkin-Elmer Guide for Atomic Absorption, 1996).

The metal concentration is calculated by using the following formula:

$$\text{Metal concentration} = \text{Dilution factor} / \text{Weight of sample}$$

In this study, dilution factor = 20ml and weight of sample = one gram in case of faecal food and soil and 1ml in case of water

The statistical analysis of the samples collected from Bahawalpur and Lahore zoo was done by using SPSS software version 21.0. The statistical analysis carried out by oneway ANOVA and non significant difference ($P > 0.05$) was present in water and soil samples of both sites however, a significant difference ($P < 0.05$) was noticed for the metals in faecal and feed samples except Zn at 0.05 significance level.

Results

The mean concentrations of heavy metals such as lead (Pb), copper (Cu), zinc (Zn), and cadmium (Cd) were detected in all samples of spotted deer (*Axis axis*) kept in Bahawalpur and Lahore zoo.

The concentrations of lead was observed as 2.2 and 0.367 µg/g in faecal samples while 0.093 and 0.033 µg/g in feed samples for both Lahore and Bahawalpur Zoo respectively. A minimum concentration was seen in water for Pb; however, in soil samples the concentration was decreasing with depth at both sites (Table I). The concentration of Cd in faecal samples was observed as 2.173 and 1.367 µg/g; however, a very small amount in feed samples 0.34 and 0.212 µg/g was noticed in Lahore zoo and Bahawalpur zoo respectively. Moreover, no Cd was detected in water samples of both sites with negligible amount in soil samples that decreased with increasing depth of soil (Table I).

The concentration of Zn in faecal samples was recorded as 5.2 and 4.2 µg/g in Lahore zoo and Bahawalpur zoo while in feed samples of both sites it was 10.53 and 9.84 µg/g, respectively. The high concentration of zinc was measured in faecal samples of Lahore zoo compared to Bahawalpur zoo. A negligible concentration was noticed in water samples of both sites (Table I). However, 2.83 µg/g at 6 inches with increasing amount of 3.31 µg/g at 12 inches and decreased concentration of 2.72 µg/g at depth of 18 inches

Table I. Concentration of heavy metals (Mean±SD) in different samples of Lahore and Bahawalpur zoo.

Samples	Lahore Zoo				Bahawalpur Zoo			
	Pb (µg/g)	Cd (µg/g)	Zn (µg/g)	Cu (µg/g)	Pb (µg/g)	Cd (µg/g)	Zn (µg/g)	Cu (µg/g)
Faecal	2.2±0.09	2.17±0.03	5.2±0.01	0.97±0.01	0.36±0.01	1.36±0.01	4.2±0.09	0.14±0.09
Feed	0.09 ±0.04	0.34±0.004	10.53±0.94	3.22±0.008	0.03±0.005	0.21±0.001	9.84±0.14	1.33±0.001
Water	0.01±0.007	Nd	0.53±0.14	0.05±0.01	0.003±0.001	Nd	0.22±0.009	0.08±0.03
Soil 6"	0.05±0.008	0.007±0.003	2.83±0.14	0.08±0.001	0.16±0.001	0.05±0.005	2.33± 2.1	0.12±0.12
Soil 12"	0.05±0.005	0.007±0.004	3.31±0.18	0.09±0.001	0.04±0.04	0.04±0.03	3.13±0.05	0.11±0.001
Soil 18"	0.04±0.02	0.004±0.0009	2.72±0.05	0.09±0.009	0.06±0.001	0.02±0.0001	4.01±0.09	0.14±0.001

were seen in soil samples of Lahore zoo but a different trend with increased concentration was noticed in Bahawalpur zoo at different depths (Table I). The concentration of Cu in faecal samples was 0.97 and 0.14 µg/g while in feed samples was 3.22 and 1.33 µg/g in Lahore and Bahawalpur zoo respectively. The amount of Cu in water samples was very low on the two study sites but a comparable difference was noticed for both sites. The trend of Cu at different depth of soil was increasing in Lahore zoo but higher amount followed by low and again high was detected in soil samples of Bahawalpur zoo.

Discussion

Heavy metals are important pollutants present in the atmosphere. Their bioaccumulation in the living tissue makes them a potential hazard for the health of living beings. Exposure to vehicular exhaust, industrial emission, and various other sources is inevitable in the day to day life and it is seen that these pollutants enter in body not only through feeding or drinking water but they can also enter in the body through inhalation when present in air (Gupta and Bakre, 2013).

Faecal matter, water, feed, and soil samples of spotted deer (*Axis axis*) in Lahore as well as Bahawalpur zoo were collected to detect exposure of heavy metals in captivity. The enclosures are located alongside the highway and it was speculated that automobile exhaust could lead to potentially higher level of metals exposure. The concentration of Pb was observed high in the faecal samples compared to water, feed and soil samples of Lahore zoo but its concentration in all samples of Bahawalpur zoo was very small. The sources of Pb are both natural and anthropogenic and excessive level of Pb can cause miscarriages (WHO, 2007; Martin and Griswold, 2009). A similar study was also used to monitor Pb contamination in wild herbivores from the protected areas of Rajasthan, India in which heavy metals can be studied using herbivore waste as a bioindicator showed Pb was present in feces due to inhalation. In the continuation of this, study was

also done in mammalian fauna of Keoladeo National Park, Bharatpur (Gaumat and Bakre, 2001; Gupta and Bakre, 2013). The higher levels of Pb in faecal samples of Lahore zoo might be due to inhalation of Pb from the air as vehicles can be considered a source of Pb in air. Stone *et al.* (2010) also reported Lahore as more polluted emerging city due to heavily contaminated air and Sughis *et al.* (2012) also reported upcoming problems such as higher systolic and diastolic blood pressure in school going children of Lahore are due to air pollution. The concentration of Cd was observed high in the faecal samples compared to feed and soil samples of both zoos however its concentration remain undetected in water samples. The higher level of Cd in faecal samples of Lahore zoo might be due to inhalation of Cd from the air as industries and human activities can be a source of air, water and soil pollution (Sathyamoorthy *et al.*, 2016). Gupta (2013) also reported parallel results as higher amounts of Cd in faecal samples compared to feed might be due to inhalation of this metal from the air. Satarug *et al.* (2003) reported 30-50 µg/day Cd intake is toxic and results in various health problems for humans.

In both Bahawalpur and Lahore Zoo, it was observed that level of Zinc in feces and feed was high at both locations. Overall, level of Zinc in different samples was also high compared to other metals such as Cd, Pb and Cu. The higher levels of Zn in faecal showed reflection of feed; however, this metal might be abundantly present in the atmosphere as production of this metal by wearing of vehicle tire reported by Nolan (2003).

The amount of Cu in faecal samples was 0.14 µg/g and 0.096 µg/g in Lahore and Bahawalpur zoo that was very low compared to amount of Cu (10.09± 1.23) in *Axis axis* of Bikaner Zoo reported by Gupta (2013) and also very high concentration for feed, water and soil samples was reported compared to present study (Gupta, 2013).

Conclusion

It was concluded that non-invasive technique is a useful method to inform heavy metal contamination of

environment in wild animals and it can be used to evaluate environmental exposure on daily basis. All the studied metals such as Pb, Cd, Zn and Cu were within range in feed samples; however, negligible concentration was present in water samples but in soil samples, different concentrations of metals at different levels of depth showed a random trend of deposition irrespective to metal type. Moreover, a higher level of environmental pollutants in Lahore zoo was noticed compared to Bahawalpur zoo; however, all the metals were found below the toxic range.

Statement of conflict of interest

The author declares there is no conflict of interest.

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