ESTIMATION OF HEAVY METAL EXPOSURE IN CAPTIVE FACILITIES OF AFRICAN LION (PANTHERA LEO)

R. Yasmeen[*], A. Raza[*], B.N. Khan[**], S. Mazhar[*], S.S. Bokhari[*], U. Rafi[*] and A.W. Qurashi[*]

[*]Department of Biology, Lahore Garrison University Sector C Phase 6 DHA Lahore,
[**] Centre for Undergraduate Studies, University of the Punjab, Lahore
[*]Corresponding author's email: roheelayasmeen@lgu.edu.pk

ABSTRACT: Conservation of wildlife is of prime importance for human beings. However, rapid industrialization, urbanization and other anthropogenic activities are polluting the environment. All these factors are responsible for human health deterioration and also affect animals by threatening and extinction of various species. The present study was conducted to detect heavy metal exposure of African lion (*Panthera leo*) found in Lahore zoo (site1) and Safari zoo (site2). Different samples such as feed (fresh meat and leftover meat), fecal, soil, and water were collected twice in a week for a month and analyzed for heavy metal contamination. The quantification of different heavy metals such as, Chromium (Cr), Lead (Pb), Nickel (Ni), Zinc (Zn) and Copper (Cu) were carried out by atomic absorption spectroscopy. The highest concentrations of heavy metals such as 42.32, 25.67, 13.13, 20.03 and 6.67 mg/Kg (site1) and 32.33, 18.67, 7.80, 13.33 and 5.34 mg/Kg (site2) for Cu, Cr, Zn, Ni, and Pb was observed respectively in soil of both sites. While, the least amount of 0.2, 0.13, 0.0, 0.37, 0.73 mg/L and 0.13, 0.26, 0.033, 0.39 and 0.66 mg/L for Cu, Cr, Zn, Ni, and Pb was noticed in water samples. All metals such as Cu, Zn, Ni were found in higher concentration in feed samples as1.23, 19.37, 0.36 mg/Kg (site1) and 16.32, 19.47 ,0.13 mg/Kg (site2) and reflecting in fecal samples except Pb and Cr that was higher in fecal samples as 1.46 and 2.2 mg/Kg (site1) and 0.85 and 0.87 mg/Kg (site2) than feed (Pb and Cr noticed as 1.3 and 0.87mg/Kg (site1) and 0.83and 0.69mg/Kg (site2)). The results were statistically analyzed using correlation and a positive correlation at p-value 0.999 with 0.01 significance level was observed for feed and fecal samples. It was finally concluded that the metallic concentration in feed was reflected in fecal samples. Furthermore, feces can be used as a good non-invasive method for the estimation of heavy metal exposure.

Keywords: Heavy metals, feces, meat, *Panthera leo,* atomic absorption spectrophotometry.

(Received 03-12-2018 Accepted 08-12-2018)

INTRODUCTION

A zoo is a place particularly built to provide conservative benefits and natural habitats to captive animals. The modern zoos are playing an important role by protecting species from extinction, educating people about conservation and providing a place for scientific research and public entertainment (Neely, 2018). However, due to socio-economic changes various factors such as industrialization, urbanization, and transportation results in air pollution (Li *et al*., 2018). Air pollutants such as dust, toxic gases and particularly heavy metals are not only spoiling our environment but also affecting the animals even those who kept in captivity. Heavy metals are critical in destabilizing the ecosystem because of their bioaccumulation in living organisms (Hermenean *et al*., 2015).

Heavy metals such as Cu, Zn, Ni, and many more are required for normal processes of the body but they become toxic when present in higher amounts (Pawar and Bhosale, 2018). Heavy metals can enter the body through various routes such as ingestion of food, drinking water, and air (Pandey and Madhuri, 2014). Heavy metals have toxic effects on the biota resulting in death of most living organisms (Iheanacho *et al*., 2017; Jaiswal *et al*., 2018). However, it depends on various different factors such as dose, route and duration of exposure, age, gender, health, genetics, and nutritional status of the particular individual (Tchounwou *et al*., 2012). Heavy metal toxicity particularly of lead and chromium are major concerns of today. The toxicity results in environmental health problems and potentially becomes dangerous due to their bio-accumulation through the food chain (Mohmand *et al.,* 2015).

Panthera leo (lion) is classified as a 'vulnerable' species by The International Union for the Conservation of Nature and Natural Resources (Watts, 2016). It is critically threatened in Pakistan (News week Pakistan, 2015). Keeping in consideration about nature, conservation and vulnerability the present study has been designed with non-invasive technique to get the estimation of heavy metals exposure on *P.leo* as the well being of this species are considered important. The objective of the study, therefore, focused on the feasibility of using fecal matter as an indicator of heavy metal exposure on daily basis because fecal matter is easy to collect and does not require killing or dissection of the animals for analysis.

MATERIALSANDMETHODS

Study area and animal selection: The two sites; Lahore Zoo (site1) and Safari Zoo (site2) were selected for the study of heavy metal concentrations in cages of *Panthera leo*. The total number of lions in Lahore Zoo was nineteen with five males, ten females and four cubs while the total number of lions in Safari Zoo was twenty-one, eight males and thirteen females.

Collection of samples: To determine heavy metal concentrations (fecal, meat, leftover meat, water, and soil) samples were collected from enclosures of lions at both sites. All samples were collected in tightly closed sterilized bottles and labeled as LZ and SZ for Lahore Zoo and Safari Zoo, respectively.

Heavy metal analysis: Heavy metal concentrations in all samples except water samples were analyzed according to the method of Dai *et al*., (2016) with few modifications by Gupta (2013). The samples were dried in hot air oven at 70℃ and one gram of each sample was taken and digested using concentrated nitric acid and perchloric acid, prepared in a 3:1 ratio. Initially, the samples were heated on a hot plate at 150°C for 30 minutes and finally temperature was increased to 180°C and a clear solution was obtained as an endpoint. This digestion was followed by the addition of 1-2 drops of hydrogen peroxide and samples were diluted with 10mL of de-ionized water. Later, they were filtered and stored in borosilicate glass tubes until further analysis.

Water samples were mixed with 3mL of HNO_3 (analytical grade) and heated until the solution becomes colorless. Due to clarity of water sample and absence of organic matter, it was followed by dilution and stored. The blank solution was also prepared using concentrated nitric acid and perchloric acid, prepared in 3:1 ratio heated without sample and later diluted and stored in the same condition along other samples. The chemical analysis of heavy metals was performed using the help of "flame atomic absorption spectrometry". The samples were analyzed under Flame Atomic Absorption Spectrophotometer (Z-8230) Polarized Zeeman atomic absorption spectrophotometer) and levels of lead (Pb), chromium (Cr), nickel (Ni), zinc (Zn), and copper (Cu) were determined at the respective wavelengths of 217, 357, 232, 214, and 324 nm respectively. The heavy metal concentrations were reported in mg/kg for fecal, soil and meat samples and mg/L for water samples.

Statistical analysis of Heavy metal: The results were statistically analyzed using the correlation coefficient at the p-value of 0.01 between heavy metal analyses of two sites using SPSS Version. 21(Zhang *et al*., 2012)

RESULTS AND DISCUSSION

The results of atomic absorption spectrophotometer showed the presence of heavy metals such as Lead (Pb), Chromium (Cr), Nickel (Ni), Zinc (Zn), and Copper (Cu) in water and soil samples of Lahore and Safari Zoo except Zn that was almost undetectable in water samples (Fig 1 & 2). Regarding the water sample, the maximum concentration of Pb was observed in both sites (site1: 0.73mg/L and site2: 0.66 mg/L) as shown in fig1. Even though, the amount of Cr and Ni in water samples of both sites were low but it was found still higher than the WHO standards (Javed and Usmani, 2013) (Fig 1). Similar results with the noticeable amount of heavy metals in water samples were reported from Kota Zoo and with considerable amount from Jaipur Zoo, India (Gupta and Bakre, 2013). It was due to change of location as Kota Zoo was surrounded by trees compared to Jaipur Zoo which was surrounded by various smelter unit, industries, and vehicle that showed air pollution affects directly to the captive sites. In the present study higher concentration of Pb in water samples of both sites was due to the contiguous environment as site 1 was urban and site 2 was open but surrounded by industrial area. The heavy metals have ability to affect living organisms both animals and plants via drinking water as reported by Gupta and Santra, (2012).

Heavy metals (Pb, Cr, Ni, Cu, and Zn) concentrations detected in soil samples were higher than those observed in water samples of both sites (Fig 1 & 2).The higher concentration of heavy metals in soil and water might be the result of atmospheric outcome as also reported by (Huang *et al*., 2015; Weldeslassie *et al*., 2018). Soil acts as long-term store, hence effectively integrating the deposition from the immediate environment (Li *et al*., 2015; Jaiswal *et al*., 2018). Pandey *et al.* (2012) reported the concentration of Pb in soil samples as 7.70 mg/Kg. This value was higher than that of both sites of the present study as 6.67mg/Kg for site1 and 5.34 mg/kg for site 2. For this, settled dust was found as one most probable reason.

The toxic affect of Lead in living organisms was reported whenever the organisms get exposed to lead and lead-containing compounds from air via inhalation, taking lead-contaminated drinks (water, milk) and eating lead-contaminated foods (fruits, meat, seafood, and grains) (Tiwari *et al.*, 2013). Rota *et al*. (2018) also reported Cd, Pb and Zn as main pollutant metals emitted from vehicles and affects the surrounding community.

Heavy metal concentration in feed (fresh and leftover meat) showed a higher concentration of Cu and

Zn. Both metals are an important component of diet but they become toxic when present in higher concentrations (Badis *et al.*, 2014). However, their concentrations were lower in the present study than the toxic limits. Samples of leftover meat that were removed from cages showed that concentrations of metals were found higher in leftover meat than fresh meat except Pb and Cr (Fig 3&5). Lead is one of the toxic heavy metal and not any recognized and useful affects of this metal has been reported in living organisms. However, its accumulation in the bodies of living organisms over time can cause various health problems (Binkowski, 2012). The known source of Pb contamination in animals are air, water, and food which they intake and possible sources of lead in air might be mining, refining, smelting, and manufacturing units (AbdEl-Salam *et al.*, 2013). The amount of Pb was lower in the present study as compared to tolerable limit (0.1 mg/Kg) as reported by (Nkansah and Ansah, 2014). However, Cr levels in the meat samples was found to be lower than the tolerable level of 1.0 mg/kg (USDA2006) and recommended levels of World Health Organization (WHO) for Cr was 1.75 mg/person/week (WHO, 2004). Moreover, higher concentrations of some metals in leftover meat showed that the settled dust in cages may be one possible reason as dust in ambient air settled in close proximity (Pavilonis *et al.*, 2015; D'Ann *et al.*, 2016; Tapia *et al.*, 2018).

In both sites, it was noticed that the concentration of Pb and Cr were found higher in fecal samples compared to feed (Fig 4). The values observed for the concentration of heavy metals in fecal samples were positively correlated with their observed values in diet (Table: 1 & 2). Different studies were also in agreement and described higher level of metals in fecal and urine excreted when animals exposed to metals and vehicular smoke via inhalation (Varsha, 2013; Chuang *et al.*, 2015; Nigra *et al.*, 2016; Yabe *et al.*, 2018; Li *et al.*, 2019). The concentrations of different heavy metals in fecal samples of lions at Bikaner zoo evaluated for Pb, Cr, Cu, and Zn were found to be 2.6, 17.8, 16.47 and 29.81mg/Kg respectively. The heavy metal concentrations in lion's fecal samples reported in Jaipur zoo for Pb, Cr, Cu and Zn were56.0±2.79, 3.19±0.11, 4.27±0.011and 40.15±0.98 mg/Kg respectively. These results were found higher than the present study (Gupta, 2013; Gupta and Bakre, 2013). Both zoos of India showed they were surrounded by heavily contaminated areas. Chemical contamination in plants and animals that lived along the roadside was also reported due to vehicle's smoke (Luo *et al.*, 2018; Rota *et al.*, 2018). The results of fecal matter in both site samples particularly for Pb and Cr showed reflection of higher concentration in the surrounding environment along meat and soil samples as site1 was actually located in the center of the city and has higher level of these metals in air. The ambient air quality of Lahore is not good and it was also categorized as second largest polluted city of Pakistan (Riaz and Ahmad, 2018). The heavy metal pollution is increasing in Pakistan due to vehicular smoke (Naz *et al.*, 2016; Shakir *et al.*, 2016; Iheanacho *et al.*, 2017). However, the most probable reasons for contamination in site 2 may be due to different activities that were observed during sampling period such as construction, paints, development of electrically operated swings in front of lion cages and heavy trucks transporting building materials. The site 2 also surrounded by the presence of different industries such as millat factory for tractor spare parts, cola drinks units, sewage drainage polluted with industrial effluents, feed mills and pipeline making units almost within five-kilometer range. Moreover, the use of feces was found as one of the best non-invasive techniques for getting an estimation of heavy metal exposure (Fig 3&4). Different studies showed that fecal matter was a best way to get estimation of heavy metal pollution (Costa *et al.*, 2013;GuptaandBakre, 2013;Gupta, 2013;Janaydeh *et al.*, 2018).The present study is also comparable to that of Bikaner zoo, which showed that food plays a significant role in heavy metal contamination in mammals while drinking water contributed to a little extent (Gupta, 2013). Zdrojewicz *et al.* (2016) for Ni poisoning while Govind and Madhuri, (2014) and Yazdankhah *et al.*(2014) reported for different trace metals such as Ni, Zn and Cu that are important for various normal processes and used to enhance immunity of living organisms when present in small amount. However, all these metals become hazardous when they exceeded from some particular limits (Vashishat and Kler, 2014) and the recommended levels of World Health Organization (WHO, 2004) for Cu and Zn was found to be 245 and 490 mg/person/Kg, respectively.

Table 1: Correlation analysis of heavy metal concentration between samples of Lahore Zoo (Site 1).

	Water	Feces	Soil	Fresh meat	Leftover meat
Water	1	-.57	-.44	-.55	-.568
Feces	-.57	1	-.33	.99**	1**
Soil	-.44	-.33	1	-.34	-.34
Fresh meat	-.55	.99**	-.34	1	.99**
Leftover meat	-.568	1**	-.34	.99**	1

**Correlation is significant at the 0.01 level (2-tailed)

Table 2: Correlation analysis of heavy metal concentration between samples of Safari Zoo (Site 2).

	Water	Feces	Soil	Fresh meat	Leftover meat
Water	1	-.740	-.45	-.81	-.798
Feces	-.740	1	-.16	.86	.94
Soil	-.45	-.16	1	.29	.127
Fresh meat	-.81	.86	.29	1	.98**
Leftover meat	-.798	.94*	.127	.98**	1

*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

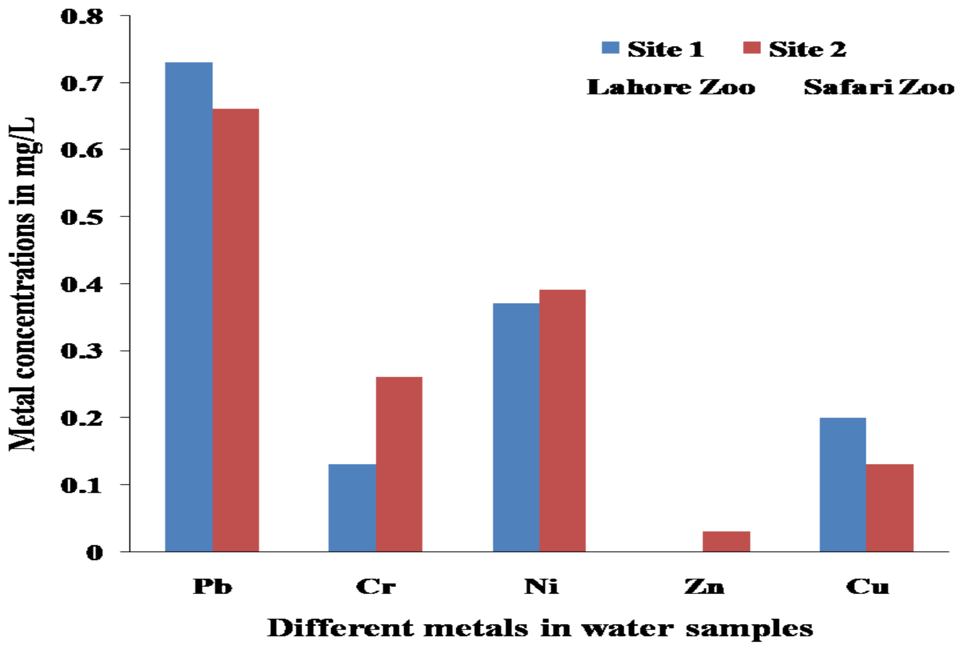

Figure-1: Concentration of different metals in water samples

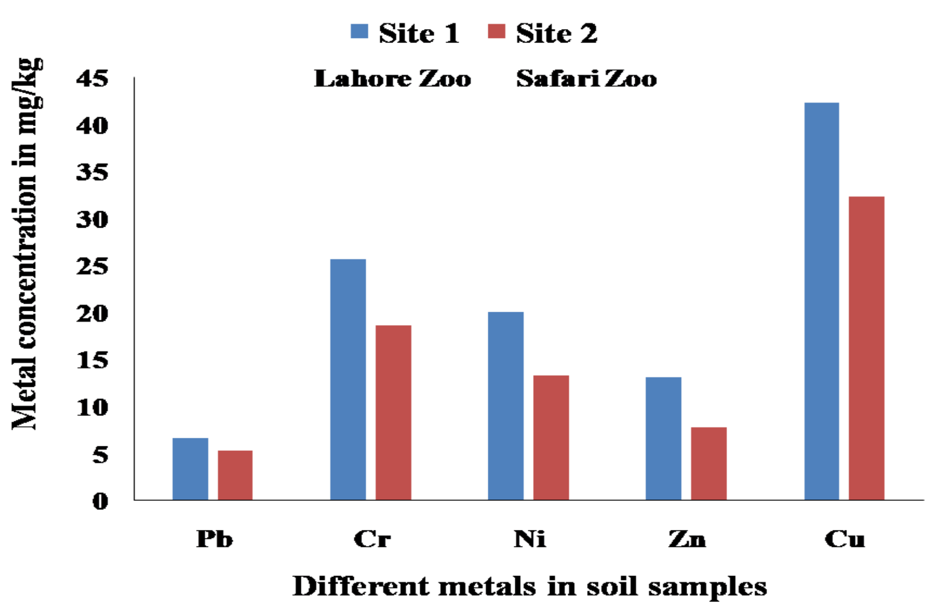

Figure-2: Concentration of different metals in soil samples

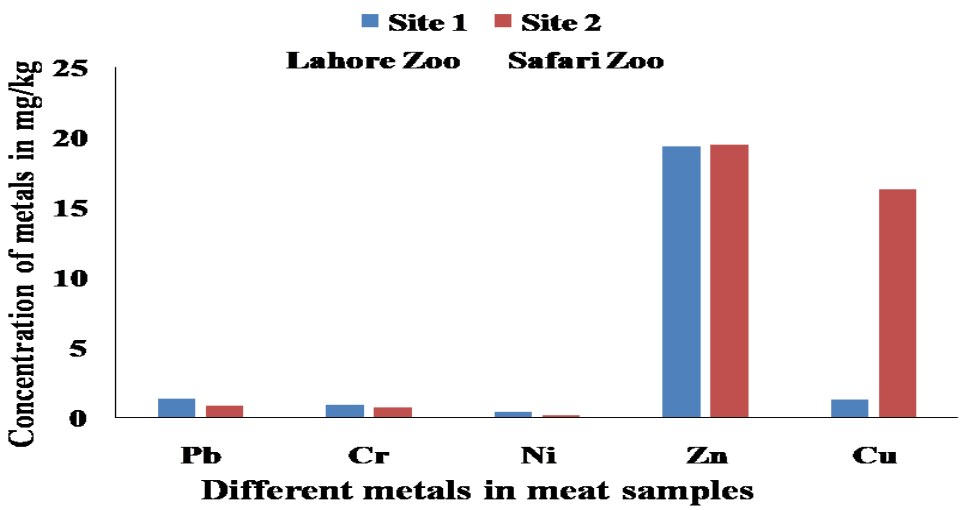

Figure-3: Concentration of different metals in meat samples

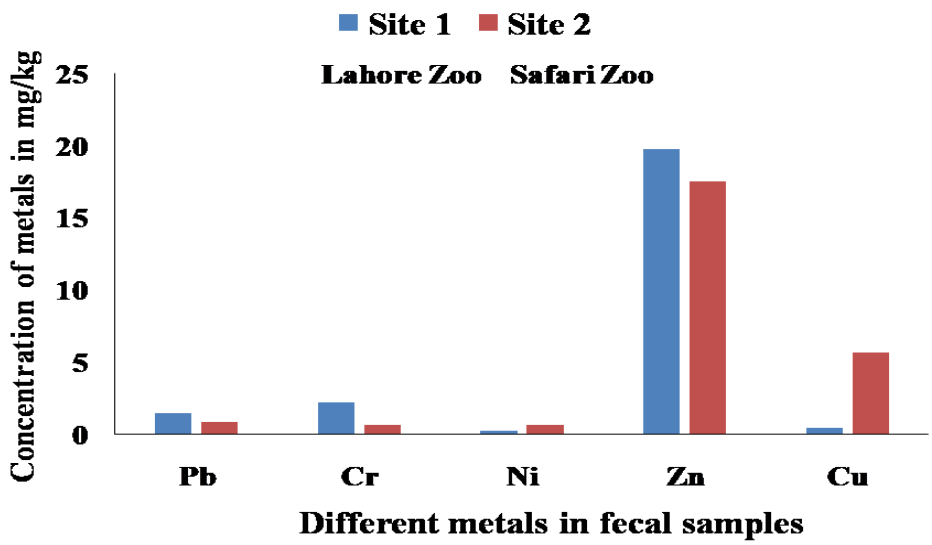

Figure-4: Concentration of different metals in fecal samples

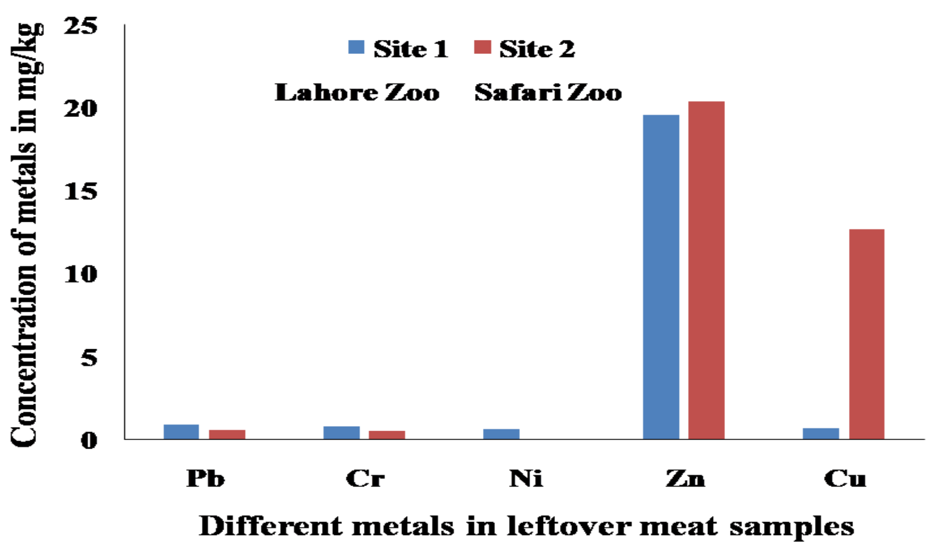

Figure-5: Concentration of different metals in leftover meat samples

Conclusion: Heavy metals in samples of both zoos showed that urbanization and heavy traffic near captive sites resulted in accumulation of pollutants in air, water and soil that ultimately effect indigenous communities and may result in species endanger.

REFERENCES

Badis, B., Z. Rachid and B. Esma (2014). Levels of selected heavy metals in fresh meat from cattle, sheep, chicken and camel produced in Algeria. Ann. Res. Rev. Biol. 4(8): 1260.

Chuang, K.J., C.H. Pan, C.L. Su, C.H. Lai, W.Y. Lin, C.M. Ma and H.C. Chuang (2015). Urinary neutrophil gelatinase-associated lipocalin is associated with heavy metal exposure in welding workers. Sci. Rep. 5: 18048.

Costa, R.A., T. Eeva, C. Eira, J. Vaqueiro and J.V. Vingada (2013).Assessing heavy metal pollution using Great Tits (Parus major): feathers and excrements from nestlings and adults. Environ. Moni. Assess. 185(6): 5339-5344.

Dai, S.Y., B. Jones and K. Lee (2016). Regulatory Science Heavy Metal Contamination of Animal Feed in Texas. J. Reg. Sci. 21–32.

D'Ann, L.W., M.C. McCormack, E.C. Matsui, G.B. Diette, S.E. McKenzie, A.S. Geyh and P.N. Breysse (2016). Cow allergen (Bos d2) and endotoxin concentrations are higher in the settled dust of homes proximate to industrial-scale dairy operations. J. Expo. Sci. Environ. Epid. 26(1): 42.

Environmental Protection Agency (EPA): Drinking Water Treatability Database for Chromium CAS Number: 7440-47-3.

Govind, P and S. Madhuri (2014). Heavy metals causing toxicity in animals and fishes. Res. J. Ani. Vet. Fis. Sci. 2(2): 17-23.

Gupta, N., D.K. Khan and S.C. Santra (2012). Heavy metal accumulation in vegetables grown in a long-term wastewater-irrigated agricultural land of tropical India. Environ. Moni. Assess. 184(11): 6673-6682.

Gupta, V (2013). Feces of captive wild mammals as bioindicator of heavy metal pollution in urban air. Int. J. Innov. Res. Sci. Eng. Technol. 2(6): 2404-2411.

Gupta, V and P. Bakre (2013).Mammalian feces as bioindicator of urban air pollution in captive mammals of Jaipur Zoo. World Environ. 3(2): 60-65.

Gupta, V and P. Bakre (2012).Metal contamination in mammalian fauna of Sariska tiger reserve, Alwar, India. J. Ecophysio. Occup. Health. 12(1-2): 43.

Hermenean, A., G. Damache, P. Albu, A. Ardelean, G. Ardelean, D.P. Ardelean and S. Keki (2015). Histopatological alterations and oxidative stress in liver and kidney of Leuciscuscephalus following exposure to heavy metals in the Tur River, North Western Romania. Ecotoxicol. Environ. Saf. 119: 198-205.

http://newsweekpakistan.com/african-lions-are-endangered-must-be-protected-u-s/

Huang, Y., T. Li, C. Wu, Z. He, J. Japenga, M. Deng and X. Yang (2015). An integrated approach to assess heavy metal source apportionment in peri-urban agricultural soils. J Hazard. Mater. 299: 540-549.

Iheanacho, E.U., J.C. Ndulaka and C.F. Onuh (2017). Environmental pollution and heavy metals. Environ. Poll. 5(5).

Jaiswal, A., A. Verma and P. Jaiswal (2018). Detrimental Effects of Heavy Metals in Soil, Plants, Aquatic Ecosystem as well as in Humans. J. Environ. Path. Tox.

Janaydeh, M., A. Ismail, H. Omar, S.Z. Zulkifli, M.H. Bejo and N.A.A. Aziz (2018). Relationship between Pb and Cd accumulations in house crow, their habitat, and food content from Klang area, Peninsular Malaysia. Environ. Moni. Assess. 190(1): 47.

Javed. M and N. Usmani (2013). Assessment of heavy metal pollution (Cu, Ni, Fe, Co, Mn, Cr, Zn) pollution in effluent dominated rivulet water and their effect on glycogen metabolism and histology Mastacembelusarmatus. Springer Plus. 2 (1):390.

Li, J., Y. Xu, L. Wang and F. Li, (2019). Heavy metal occurrence and risk assessment in dairy feeds and manures from the typical intensive dairy farms in China. Environ. Sci. Poll. Res. 1-11.

Li, M., C. Li and M. Zhang (2018).Exploring the spatial spillover effects of industrialization and urbanization factors on pollutants emissions in China's Huang-Huai-Hai region. J. Clean Prod. 195(1):154-162.

Li, N., Y. Kang, W. Pan, L. Zeng, Q. Zhang and J. Luo (2015). Concentration and transportation of heavy metals in vegetables and risk assessment of human exposure to bioaccessible heavy metals in soil near a waste-incinerator site, South China. Sci. Total. Environ. 521(1): 144-151.

Luo, J., M.J. Barth and K. Boriboonsomsin (2018). Vehicle Routing to Mitigate Human Exposure to Traffic-Related Air Pollutants. 21st Int. Conf. Intelli. Transport, Sys. (ITSC) 2765-2770.

Mohmand, J., S.A.M.A.S. Eqani, M. Fasola, A. Alamdar, I. Mustafa, N. Ali and H. Shen (2015). Human exposure to toxic metals via contaminated dust:

Bio-accumulation trends and their potential risk estimation. Chemosphere, 132: 142-151.

Naz, N., F.H. Nasim and M. Ashraf (2016). Vehicular emissions and ambient air quality along road side in Rahim Yar Khan, Pakistan. Pak. J. Sci.. 68(3).

Neely, J.C. (2018). Zoo Story: Narrative, Virtue Ethics, and Deconstructing Dualisms in the Journalism of Thomas French. J. Media Ethics, 33(2): 80-91.

Nigra, A.E., A. Ruiz-Hernandez, J. Redon, A. Navas-Acien and M. Tellez-Plaza (2016). Environmental metals and cardiovascular disease in adults: a systematic review beyond lead and cadmium. Curr. Environ. Health Rep. 3(4): 416-433.

Nkansah, M.A. and J.K. Ansah (2014). Determination of Cd, Hg, As, Cr and Pb levels in meat from the Kumasi Central Abattoir. Int. J. Sci. Res. 4(8): 1-4.

Pandey, G. and S. Madhuri (2014). Heavy metals causing toxicity in animals and fishes. Res. J. Ani. Vet. Fishery Sci. 2(2): 17-23.

Pandey V.C. (2012). Phytoremediation of heavy metals from fly ash pond by Azollacaroliniana. Ecotoxicol. Environ. Saf. 82: 8-12.

Pavilonis, B.T., P.J. Lioy, S. Guazzetti, B.C. Bostick, F. Donna, M. Peli and P.G. Georgopoulos (2015). Manganese concentrations in soil and settled dust in an area with historic ferroalloy production. J. Exp. Sci. Environ. Epid. 25(4): 443.

Pawar, P.R. and S.M. Bhosale (2018). Heavy Metal Toxicity, Health Hazards and their Removal Technique by Natural Adsorbents: A Short Overview.

Riaz, R. and K. Hamid (2018). Existing Smog in Lahore, Pakistan: An Alarming Public Health Concern. Cureus, 10(1).

Rota, E., B. Braccino, R. Dei, S. Ancora and R. Bargagli (2018). Organisms in wall ecosystems as biomonitors of metal deposition and bioavailability in urban environments. Environ. Sci. Poll. Res. 25(11): 10946-10955.

Shakir, S.K., A. Azizullah, W. Murad, M.K. Daud, F. Nabeela, H. Rahman, and D.P. Hader (2016). Toxic metal pollution in Pakistan and its possible risks to public health. Rev. Environ. Contam. Toxicol. 242: 1-60.

Tapia, J.S., J. Valdes, R. Orrego, A. Tchernitchin, C. Dorador, A. Bolados, and C. Harrod (2018). Geologic and anthropogenic sources of contamination in settled dust of a historic mining port city in northern Chile: health risk implications. Peer J. 6:4699.

Tchounwou, P.B., C.G. Yedjou, A.K. Patlolla and D.J. Sutton (2012). Heavy metal toxicity and the environment. Mol. Clin. Environ. Toxicol. 133-164.

USDA (2006). Foreign Agricultural Services GAIN Report; Global GAIN Report No. CH6064, Chinese People’s Republic of FAIRS products. Specific maximum levels of contaminants in foods, Jim Butterworth and Wu Bugang. 1-60.

Vashishat, N. and T.K. Kler (2014). Birds as bioindicators of heavy metal pollution. Agrolook.

Watts, S (2016). Protection of the African lion: a critical analysis of the current international legal regime. *PER:* Pot chef stroomse Elektroniese Regsblad, 19(1): 1-41.

Weldeslassie, T., H. Naz, B. Singh, and M. Oves, (2018). Chemical Contaminants for Soil, Air and Aquatic Ecosystem. In Modern Age Environmental Problems and their Remediation 1-22p.

Yabe, J., S.M. Nakayama, Y. Ikenaka, Y.B. Yohannes, N. Bortey-Sam, A.N. Kabalo and M. Ishizuka (2018). Lead and cadmium excretion in feces and urine of children from polluted townships near a lead-zinc mine in Kabwe, Zambia. Chemosphere. 202: 48-55.

Yazdankhah, S., K. Rudi and A. Bernhoft (2014). Zinc and copper in animal feed development of resistance and co-resistance to antimicrobial agents in bacteria of animal origin. Microbiol. Ecol. Health dis. 25(1): 25862.

Zdrojewicz, Z., E. Popowicz and J. Winiarski (2016). Nickel - role in human organism and toxic effects. Polskimerkuriuszlekarski: *organ Polskiego Towarzystwa Lekarskiego*, 41(242):115-118

Zhang, F., Y. LI, and M. Yang (2012). Content of heavy metals in animal feeds and manures from farms of different scales in Northeast China. Int. J Environ. Res. Public Health. 9(8): 2658–2668.